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Phenological and yield response of primed carrot (*Daucus carota* L.) seeds under deficit irrigation

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Key words: Carrot, irrigation interval, marketable yield, physiological changes, priming.

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Abstract: Seedling emergence and stand establishment of carrot seeds are often slow and erratic which results in low productivity. Poor seed quality together with lack of pre-sowing seed treatments and improper irrigation management can be mentioned as the major factors that influence the yield and productivity of carrot. The present study was carried out with the objective to evaluate the effects of different seed priming techniques on early seedling establishment, growth and yield of carrot (*Daucus carota* L. cultivated variety Nantes) exposed to different irrigation intervals, under field conditions at Gerado, South Wollo, Ethiopia. Four seed priming treatments (no priming, hydro priming, halo priming and hormonal priming) and three irrigation intervals (4, 7 and 10 days) were combined as factorial RCBD in split plot arrangement with three replications. The irrigation intervals were assigned to main plots and the seed priming techniques to sub plots. Result indicated that the interaction effects of priming techniques and irrigation intervals significantly affected the phenological and yield parameters. Distilled water treatment in seven and ten days irrigation interval recorded the highest marketable carrot root yields of 33.73 t h^{-1} and 30.63 t h^{-1}, respectively. Hence, hydro priming and seven days irrigation interval can be recommended for the production of carrot in the study area and similar agro-ecologies. Given the promising results obtained, further repetitions of the study are recommended to validate the use of these techniques in further locations and in different seasons.

1. Introduction

Carrot (*Daucus carota* L.) is a crop that belongs to the family *Apiaceae* previously *Umbelliferea* (Thiviya *et al.*, 2021). Carrot is among the top ten most economically important vegetable crops in the world in terms of areas of production and market value (Simões *et al.*, 2010). Its yield can range from 30 to 100 t ha^{-1} in major carrot growing countries of the world (Tegen and Jembere, 2021).

Carrot is one of the major root vegetable crops produced in Ethiopia. It

is nowadays cultivated predominantly as a cash crop throughout the country, for sale to urban markets, including Hotels and Restaurants as indicated by Getachew and Mohammed (2012). According to CSA (2022) about 6,759.92 ha has been covered by carrots with a total production of 31,671.6 tons and productivity of 4.7 t ha^{-1}. In 2022, the area coverage of carrots increased by 30% compared to the production year of 2021 whereas, in the Amhara region about 1,619.58 ha has covered by carrots with a total production of 9,145.8 tons and productivity of 5.6 t ha^{-1}. The productivity of carrots in Ethiopia is very low (4.7 t ha^{-1}) compared to other countries which can be up to 100 t ha^{-1} in major carrot-growing countries of the world as reported by CSA (2022).

Carrot has remarkable nutritional and health value and is considered as a rich source of carotenoids, phenolic compounds, polyacetylenes, and vitamins (Alhariri and Boras, 2020; Glowka *et al.*, 2021). The health benefits and public awareness of nutritional health security are positively influencing the demand and consumption of carrots by consumers and by the nutraceutical-based industry (Selvakumar *et al.*, 2019).

Heterogeneous, asynchronized and lower rates of seed germination are major challenges in *Apiaceae* (*Umbelliferae*) family and have a major impact on final yield and quality especially for vegetable crops established by direct seeding such as carrot and parsley (Mozumder and Hossain, 2013). Improvement in seeds' homogenous, longevity and germination speed are major concerns for carrot growers and the seed agribusiness sector. Recently, different pre-sowing seed treatments have been proposed to improve seed vigour, uniformity and seedling emergence (Sagvand *et al.*, 2022). The crop is usually established by direct seeding and therefore, low soil moisture content may lead to poor crop stand (Mahmood-Ur-Rehman *et al.,* 2020). Low soil moisture content delay or inhibit seed germination in the field, reduce uniformity of seedling performance, total stand establishment and ultimately reduce the yield of carrot. In addition, before sowing the seeds, it is essential to ensure that the soil is adequately moist. This can be achieved by irrigating the field a few days before planting. The moisture content should be sufficient for the seeds to absorb water and germinate.

The production and productivity of carrot in the study area is low mainly due to low quality seeds and poor cultural practices including improper irrigation intervals. Carrot producers mainly use seeds stored for long periods which hardly germinate in the field. The germination is not uniform and fails to establish a homogeneous crop stand especially under limited irrigation intervals. Different seed priming techniques help to enhance seed quality thereby improving seedling emergence percentage and uniformity even under stress conditions. Even though a number of researches have been done on seed priming (Pereira *et al.*, 2009; Paparella *et al.*, 2015), there is no single method of seed priming technique that can be best suited to all crops. Thus, it's of paramount importance to examine the different priming techniques to enhance the quality of carrot seeds. Further, water requirement studies for proper irrigation scheduling of carrot also has great importance. However, information regarding the efficient use of irrigation water for improved growth and yield of carrot has also lacking in Ethiopia. Scarcity of irrigation water is an acute problem for successful crop production in Ethiopia. Hence the need for more efficient utilization and management of scarce irrigation water is crucial. Therefore, this research was initiated to investigate the use of different seed priming techniques on seedling emergence, growth and yield of carrot under different irrigation frequencies in South Wollo, Ethiopia.

2. Materials and Methods

Description of experimental site

The experiment was conducted at Gerado, mid-altitude of south Wollo administrative zone, Amhara regional state, Ethiopia during 2020/21 cropping season. The study area is geographically situated 11 12' 00'' north and 39 42' 00'' east with an elevation between 2200-2800 m a.s.l. (Fig. 1) The general climate is midland with a mean annual rainfall ranging

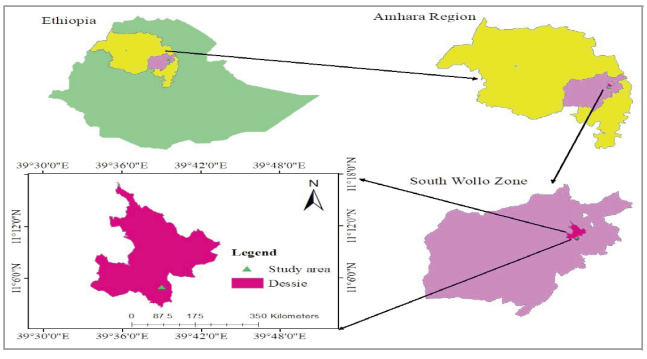

Fig. 1 - Geographical location of the study area.

from 900 mm to 1000 mm and mean annual temperature between 15°C to 20°C (Tamru, 2006). The major soil type in the area is sandy-loam. Gerado has three permanent rivers which have the potential to irrigate throughout the year. These are Yito Medehanialem, Negewoliy and Gerado. In the study area the most cultivated crops are maize, teff, wheat and barley. Vegetable crops such as carrot, cabbage, tomato, and potato are cultivated using irrigation water from the nearby rivers (Bahir *et al.*, 2015).

Experimental material

Carrot (*Daucus carota* var. *Nantes)* seed (250 g), were obtained from vegetable seed providing enterprise in Dessie city andused as a test crop since it is adapted to the experimental area and preferred by most small- holder farmers and consumers. Nantes variety has orange colour and cylindrical roots with a blunt end and strong leaves. It has a wide adaptability and grows well at altitudes ranging from 1600-2400 m a.s.l with annual precipitation of 760-1010 mm and can be produced all year round both under rain-fed and irrigation condition (Hubbard *et al.*, 2012).

Treatment and experimental design

This study applied two treatments concerning priming techniques and irrigation levels. In total four techniques of priming; none primed/control (NP), potassium nitrate (KNO_3), gibberelic acid (GA_3), and distilled water (DW) where seeds were dipped in the respective solutions 12 priming hours. Irrigation treatment consisted of three irrigation intervals: four days interval (I4), seven days interval (I7) and ten days interval (I10).KNO_3 was used at a concentration level of 50mM (Lara *et al.*, 2014) and GA_3 was used at a concentration level of 0.05 mM (Kwon *et al.*, 2020). The ratio (W/V) of seed: priming solutions was 1:5 (g/ml) (Ruan *et al.*, 2002).

The experiment was laid out in Randomized Complete Block Design (RCBD) in split plot arrangement with three replications. There were 3 main plots in each replication and each main plot was further divided into 4 sub plots where irrigation intervals assigned as main plots and the priming techniques was assigned to sub-plots. The gross area of the experimental site was 240 m^2 (30x8) and the net area was 103.68 m^2. The area of a single plot was 2.88 m^2 (1.6x1.8 m). Spacing between main plots and sub-plots were 1m and 0.5m respectively. Blocks were separated by 1.5 m. The seeds were planted on rows with spacing of 5 cm between plants and 20 cm between rows. Each experimental plot had 7 rows with 36 plants per row. The outer single rows at both sides of the plot and one plant at both ends of the rows were considered as border plants. All management activities except irrigation and priming techniques were applied uniformly in all plots of the experiment.

Experimental procedure and management of plants

At first, seeds were superficially sterilized with sodium hypochlorite solution (2%) for five minutes to sterilize fungal agents and were then thoroughly wash with distilled water and air dried (Farooq *et al.*, 2005). Seed samples of Nantes variety was then divided in to three for each priming media. After that, seeds except the non - primed (control) were fully immersed in different priming medias (distilled water, GA_3 and KNO_3) on Petri dishes for 12 priming hours (Abnavi and Ghobadi, 2012). After the priming application, all seeds were removed from the priming media at the same time and then rinse thoroughly with distilled water, dried on paper towels at room temperature and ventilated until they regained their original weight (Giri and Schillinger, 2003).

CROPWAT version 8.0 was used to calculate the amount of water required by carrot crop and water was applied using calibrated watering can to bring the water requirement of each interval days for the treatments. Crop water requirement (CWR) is the total water needed for evapotranspiration, from planting to harvest for a given crop in a specific climate regime when soil does not limit plant growth and crop yield (Ouda *et al.*, 2015). Considering this the CWR was determined by the formula developed by FAO as follows.

$$ETc = Kc \times ET_0 \qquad \text{Eq. 1}$$

where ETc is crop potential evapotranspiration (mm/unit time), Kc is crop coefficient (Influence of crop type and growth stage) and ET_o is reference evapotranspiration (mm/unit time) [Influence of climate].

The effect of climate on crop water requirements is given by the reference crop evapotranspiration (ET_o) which is defined as the rate of evapotranspiration from an extensive surface of 8 to 15 cm tall, green grass cover of uniform height, actively growing, completely shading the ground and not short of water. FAO (2005) defined the crop potential evapotranspiration (ET_o) for a given crop as:

$$ET_0 = \frac{0.408\Delta(Rn - G) + \gamma\frac{900}{T+273}U2(es - ea)}{\Delta + \gamma(1 + 0.34U2)} \quad \text{Eq. 2}$$

where ETo is the reference evapotranspiration (mm day^{-1}), Rn is the net radiation (MJ m^{-2} day^{-1}), G is the soil heat flux density (MJ m^{-2} day^{-1}), T is the mean daily air temperature at 2 m height (^oC) Δ is the slope of the saturated vapour pressure curve (kPa $^oC^{-1}$), γ is the psychrometric constant (66 Pa $^oC^{-1}$), es is the saturated vapour pressure at air temperature (kPa), ea is the prevailing vapor pressure (kPa), and U_2 is the wind speed measured at 2 m height (m s^{-1}).

Based on the above formula water requirement of carrot was 402.4 liter per m^2 of land for all growing season. The gross and net plot sizes were 2.88 m^2 and 2.63 m^2, respectively. Therefore, the total water requirement of each plot was 1,158.912 liters. Each treatment was irrigated with equal amount of total irrigation water during the growth period, which was calculated based on CROPWAT 8 software. Accordingly, the amount of irrigation water applied by three levels irrigation intervals was different, as the irrigation interval increases, the frequency of water application decreases leading to lesser amount of water applied and vice versa. Thus, 46.36 litres of water were supplied within the irrigation interval of 4 days, 82.79 litres in the irrigation interval of 7 days, and 115.89 litres in the irrigation interval of 10 days. The amount of water required for each interval was obtained by dividing the total water requirement of a single plot by the number of irrigation applications over the total growing season).

To determine the baseline of irrigation interval to set up the treatment, input data for the CROPWAT model were obtained from the National Meteorological Services Agency, Soil laboratory results and FAO publications. Metrological data of ten years which were collected from Ethiopian metrology agency, north eastern region, Kombolcha station were used.

Soil analysis of the experimental site

Soil samples were collected from the experimental site before treatment application at a depth of 30 cm in a zigzag pattern from nine randomly selected points by using auger. The collected samples were air dried, mixed and made a composite representative sample of 1 kg for analysis. The composite sample was packed in a polythene bag, labelled and taken to Dessie Soil Testing Laboratory. Soil analysis data on soil texture, pH, organic matter, field capacity, permanent wilting point and organic carbon of the soil were analyzed as shown in (Table 1).

Table 1 - Physical and chemical properties of the soil in the study area

Soil properties	Data
Organic matter (%)	4.13
Organic carbon	2.4
pH	7.02
Texture	
Sand (%)	45
Silt (%)	13
Clay (%)	42
Class	Sandy clay
Field capacity (vol. %)	39
Permanent wilting point	27

Data collection and statistical analysis

Phenological, growth, yield related parameters and yield were collected from net plot area using standard procedures. Data were subjected to two-way analysis of variance (ANOVA) using R-software based on Agricole statistical package. The mean separation was carried out using least significant difference (LSD) at probability level based on the results of ANOVA analysis. The detailed procedures were as follows:

Emergence percentage (%). After emergence of first seedling of every treatment, the numbers of emerged seedlings were counted daily up to 14 days after sowing. Emergence percentage was calculated as:

$$\text{Emergence (\%)} = \frac{\text{total number of emerged seedling}}{\text{total seeds sown}} \text{ x } 100$$

Mean emergence time (count). It was calculated according to (Ellis and Roberts, 1980):

$$MET = (\Sigma n \text{ x } D)/\Sigma n$$

where n is the number of seeds emerged at day D, and D the number of days since the start of emergence test (sowing).

Days to 90 % maturity (days). It was measured by counting the number of days elapsed from date of sowing to the date when 90% of the plant in each plot attained physiological maturity and used for further analysis. Carrot plants were physiologically matured when the leaf color turns in to yellow and roots are at harvestable size and the crown attained a

diameter ranging from 2-3.8 cm diameter as described by UNECE (2018).

Marketable root yield (t ha^{-1}). Carrot roots, which are free from mechanical damages, disease and insect pest attack and sizes (>50 g) were considered as marketable (UNECE, 2018). The weight of such carrots harvested from the net plot area was weighed using scale and expressed as ton per hectare.

Unmarketable root yield (t ha^{-1}). Carrot roots which are diseased, insect pest damaged, cracked and under sized (<50 g) was considered as unmarketable as described by UNECE, 2018. The weight of such carrots harvested from the net plot area was weighed using scale and expressed as ton per hectare.

Total root yield (t ha^{-1}). It was obtained by summation of marketable and unmarketable yields and then converted to hectare basis and expressed in t ha^{-1}.

3. Result and Discussion

Phenology

Emergence percentage. Increasing the frequency of irrigation as well as treating seeds with different priming solution increased the emergence percentage of carrot plants. In the interaction effect, the results showed that, decreasing irrigation interval along with priming techniques improved the emergence percentage of carrot plants. Therefore, the highest emergence percentage of carrot (83%) was observed by the treatment combination of four days irrigation interval and distilled water treatment. The lowest EP values were obtained using 10 days of irrigation interval for all the priming treatments, with minimum value in non-primed seeds (46.67%) (Table 2).

The highest emergence percentage of carrot plants in treatments receiving short irrigation intervals combined with pre-sowing seed treatment might be due to priming-induced changes in biochemical contents of the seeds, membrane integrity and enhanced physiological activities during seed germination and available moisture which is required for germination (Alam *et al.*, 2013). On the other hand, the lowest emergence percentage recorded from longer intervals might be due to moisture deficiency. The result of this study was in agreement with Selvarani and Umaran (2011), who found that hydro priming is the best priming technique for *Daucus carota* seeds. Dessalew *et al.* (2022) also reported that Halo, Hydro and Hormonal-priming techniques improved germination, seedling growth, seedling vigour and seed yield of carrot (*Daucus carota*).

Table 2 - Interaction effect of seed priming and irrigation interval on phonological parameters of carrot

Treatment combinations		Emergence (%)	Mean emergence time (Days)	DM
Irrigation interval	Priming treatment			
4 days	Gibberelic acid	77.67 b	11.33 f	99.33 f
	Distilled water	83.00 a	10.7 f	97.00 f
	None primed	76.33 c	12.96 bc	104.57 cde
	Potassium nitrate	77.67 b	12.3 d	103.90 de
7 days	Gibberelic acid	69.00 bc	13.00 bc	103.37 de
	Distilled water	75.67 ab	11.40 ef	102.20 e
	None primed	56.00 d	13.10 b	105.40 bcd
	Potassium nitrate	66.33 c	12.33 cd	104.80 bcd
10 days	Gibberelic acid	57.00 d	13.10 b	107.00 abc
	Distilled water	55.33 d	13.0 b	106.57 bc
	None primed	46.67 e	13.93 a	109.00 a
	Potassium nitrate	55.33 d	13.16 b	107.37 ab
LSD (5%)		0.07	0.66	2.41
CV (%)		6.50	3.40	1.40
SE+/-		0.03	0.42	1.40

Means with the same letter/s in column are not significantly different; * = significant (p<0.05); CV = Coefficient variance; LSD = least significant difference; SE= standard error, DM= Days to 90% maturity (Days).

Similarly, Mehri (2005) reported that the maximum germination percentages were obtained from water treatment while the least value was recorded from non-primed seeds.

Mean emergence time. Treating seeds with different soaking chemicals as well as frequent irrigation generally reduced the spread of emergence times. The different priming techniques significantly decreased the mean emergence time across the increasing frequency of irrigation as compared to control. The shortest mean emergence time (10.70 days) was recorded from seeds treated with distilled water and irrigated at four days interval. However, the combined effect of longest irrigation interval (ten days) with unprimed seeds recorded the longest mean emergence time (Table 2).

The significant reduction in emergence time derived from the combination of seed priming with frequent irrigation intervals might be due to the completion of pre-germinative metabolic activities during the priming process (Bourioug *et al.*, 2020). Primed seeds germinated soon after planting compared to untreated dry seeds since seed priming stimulates an array of biochemical changes such as hydrolysis of starch, activation of enzymes and breaking dormancy in the seed (Patel and Rai, 2018; Tania *et al.*, 2020). Furthermore early reserve breakdown and reserve mobilization might also be the cause of significant reduction in emergence time due to readily available assimilate during germination (Farooq *et al.*, 2005; Farooq *et al.*, 2006).

The results are in line with Alhariri and Boras (2020) and Mahmood-Ur-Rehman *et al.* (2020) who reported that seeds treated with different chemicals germinated faster than untreated seeds (control). Moreover, Alam *et al.* (2013) found that unprimed spinach seeds took longer to emerge compared to primed seed. Synchronized emergence of primed seeds can insure a vigorous and better crop stand with rapid canopy development, giving plants a preliminary advantage over weeds resulting in increased weed competitiveness (Dhage and Anishettar, 2007; Raj and Syriac, 2017; Juraimi *et al.*, 2020). Moreover, Safiatou (2012) reported that seed priming improved the competitive ability of a crop against weeds, and faster emergence along with increased vigour of a primed stand is the key factors for tolerating weeds.

Days to 90% maturity. Seed soaking in different priming chemicals along with frequent irrigation resulted in lowest mean number of days to reach 90% maturity. The result of interaction effect showed that, the earliest days to maturity (97.00 days) was recorded from the treatment combination of four days irrigation intervals with distilled water treatment which was statistically similar to the combined effect of gibberelic acid seed treatment and four days irrigation interval (99.33 days). The longest days to maturity (109.00 days) was recorded on unprimed seeds with the longest irrigation interval (Table 2).

The accelerated maturity of carrot roots irrigated with frequent irrigation interval might be associated with easily uptake of nutrients as the available water helps the plant to dissolve the nutrients and move through transpiration pull which in turn helps carrot roots to mature early. In addition, early emergence due to priming can contribute to early maturity. Plants which emerge early can reach maturity earlier. The results of the present study are in agreement with the findings of Reid and Gillespie (2017), who observed accelerated maturity of carrot roots with frequent irrigation intervals. Similarly, Safiatou (2012), Singh *et al.* (2015) and Aluko *et al.* (2020) also reported that compared to unprimed seeds, primed seed took significantly fewer days to emerge and reach maturity.

Yield

Marketable yield. Distilled water treatment consistently increased marketable yield of carrot similarly, increasing the duration of irrigation interval up to seven days intervals hasincreased the marketable yield of carrot. In the interaction effect, the highest marketable yield (33.73 t ha^{-1}) was recorded from seeds soaked in distilled water and irrigated in every seven days interval followed by seeds primed with distilled water and irrigated at ten days interval with the values of (28.30 t ha^{-1}) which was statistically similar with that of plants grown in the treatment combination of four days irrigation interval with distilled water treatment (27.96 t ha^{-1}). On the other hand the lowest (16.53 t ha^{-1}) marketable carrot yield was obtained from the combination of unprimed seeds with ten days irrigation interval (Table 3).

The reason for higher marketable yield from primed seeds and irrigated at seven days irrigation interval might be due to uniform and vigorous seedling growth, well-developed root system and efficient subsequent growth with lesser competition for nutrient and water that eventually led to higher yield. The lower marketable yield resulted from non-primed seeds might be associated with suboptimal uniformity at emergence resulting in poor uniformity

Table 3 - Interaction effect of seed priming techniques and irrigation interval on yield parameters of carrot

Treatment combinations		Total root yield (t ha^{-1})	Marketable root yield (t ha^{-1})
Irrigation intervals	Priming techniques		
Four days intervals	Gibberelic acid	29.03 cd	26.76 cd
	Distilled water	29.23 bc	27.03 bcd
	None primed	23.33 f	20.66 f
	Potassium nitrate	27.73 cde	25.03 cde
Seven days intervals	Gibberelic acid	29.53 bc	27.96 bc
	Distilled water	35.23 a	33.73 a
	None primed	24.46 ef	22.06 ef
	Potassium nitrate	28.90 cd	27.36 bcd
Ten days intervals	Gibberelic acid	27.63 cde	25.63 cde
	Distilled water	32.53 ab	30.63 ab
	None primed	19.33 g	16.53 g
	Potassium nitrate	25.73 def	24.03 def
LSD (5%)		3.08	3.23
CV (%)		5.40	6.30
SE+/-		1.51	1.62

Means with the same letter/s in column are not significantly different; * = significant ($p<0.05$); CV = Coefficient variance; LSD = Least significant difference; SE= standard error.

in plant size. On the other hand longer irrigation interval with non- primed seed resulted in carrot roots which are unfit for the market. Similar studies were made by Knox *et al.* (2012) who reported that supply of adequate water was critical for high quality vegetable production. The current results are supported by the findings of Govinden-Soulange and Levantard (2008), Alam *et al.* (2013) and Castañares and Bouzo (2018) who reported that seed priming is a mean to improve early flowering, maturity time and yield of a crop due to early seedling growth.

Total carrot root yield. The interaction effect of irrigation interval and priming techniques significantly impacted total carrot root yield. The highest total fresh root yield of carrot (35.23 t ha^{-1}) was recorded from distilled water seed treatment combined with seven days irrigation interval which was statistically similar with total yield obtained from the combination of ten days irrigation interval and distilled water treatment (32.53 t ha^{-1}) followed by the average fresh total root yield obtained from the interaction effects of gibberelic acid with seven days irrigation interval (29.53 t ha^{-1}). On the other hand the lowest (19.33 t ha^{-1}) total fresh root yield of carrot was recorded for plants produced from non-primed seeds and irrigated at ten days interval (Table 3).

The increased total root yield due to priming and seven days irrigation interval might be due to early seedling growth, and improved plant stands with the benefits of priming. Moreover, seven days irrigation interval produced higher yield because of sufficient soil moisture in the root zone which enhanced the uptake and assimilation of nutrients. This result was in agreement with the finding of Hamma *et al.* (2012) who reported that optimum irrigation interval significantly produced higher plant height, weight of individual root and yield per hectare of carrot.

4. Discussion and Conclusions

The results of the present study showed that phenological and yield related parameters of carrot plant were significantly influenced by both seed priming techniques, irrigation intervals and their interaction. Frequent irrigation intervals led to higher marketable root yield due to the increment in the percentage emergence, decrease in the mean emergence time and in the number of days to reach 90% maturity. The highest marketable carrot root yield 33.73 t ha^{-1} was obtained combining distilled water treatment and seven days irrigation interval.

Based on the results of the present study, plants

obtained from distilled water primed seeds and irrigated at seven days interval recorded the shortest days to mean emergence time, day to maturity and, highest marketable root yield of carrot which can be recommended for economical production of carrot in the study area and areas with similar agro-ecologies. Future research will focus on studying the effect of different concentrations of priming solutions and the duration of seed priming on seedling emergence, growth and yield.

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Salicylic acid and iron-oxide nanoparticles improved the growth and productivity of ajowan under salt stress

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Key words: Foliar application, root growth, salinity, seed filling, yield parameters.

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Abstract: Two factorial experiments with randomized complete block design in three replicates were conducted in a greenhouse at the University of Tabriz to investigate the individual and combined effects of SA and Fe_2O_3-NPs spray (1 mM and 3 mM, respectively) on cations contents, root and shoot growth, seed filling and yield parameters of salt-stressed ajowan plants (0, 4, 8 and 12 dS m^{-1} NaCl; as non-saline and low, moderate and high salinities, respectively). Salt stress enhanced Na^+ contents and reduced K^+ and Ca^{2+} contents, and K^+/Na^+ and Ca^{2+}/Na^+ ratios, leading to a reduction in root and shoot growth, particularly under high salinity. Reduction in plant growth parameters under salt stress had a negative impact on yield components and seed yield of ajowan. These deleterious impacts of salinity on plants were largely overcome by foliar treatments, particularly by SA + Fe_2O_3-NPs. The improvement of seed yield by these treatments was highly correlated with enhanced root and shoot growth, seeds per plant, and 1000-seed weight, especially under moderate and high salinities. Thus, the simultaneous application of SA and Fe_2O_3-NPs was the best foliar treatment for enhancing the growth and productivity of ajowan plants under normal and saline conditions.

1. Introduction

Growth and development of plants are constantly influenced by various environmental stresses including salinity (Sarker and Oba, 2019). Salt stress triggers many cellular events, causing physiological, biochemical and eventually morphological alterations. This stress chiefly causes ionic toxicity by enhancing Na^+ concentration in plant cells, which ultimately prevents the acquisition of essential nutrients, ionic homeostasis and cell metabolism (Nikpour-Rashidabad *et al.*, 2022). Salinity not only causes cellular water imbalance, osmotic stress and abscission, but also significantly influences different photosynthetic enzymes and gas exchange parameters (Lotfi *et al.*, 2020; Rasheed *et al.*, 2020). Salt toxicity may also lead to oxidative stress due to the physiological imbalance between the generation and scavenging of

reactive oxygen species (ROS) (Ghassemi-Golezani and Abdoli, 2022 a). Elevated ROS may cause the oxidation of proteins and membrane lipids and also impair cellular redox homeostasis. A stress-induced decline in plant growth and productivity is a common phenomenon in many plant species, which is most likely attributed to the changes in plant physiology, and metabolism (Ghassemi-Golezani *et al*., 2021; Ghassemi-Golezani and Rahimzadeh, 2022) and phenology attributes (Kazan and Lyons, 2016). The major negative impacts of salinity on root growth have been formerly confirmed in *Portulaca oleracea* (Kafi and Rahimi, 2011), *Mentha spicata* (Chrysargyris *et al*., 2019), *Oryza sativa* (Chang *et al*., 2019) and *Jatropha curcas* (Abrar *et al*., 2020), *Brassica napus* (Ghassemi-Golezani and Abdoli, 2022 b) plants. Therefore, salt toxicity is an escalating problem in different agricultural systems worldwide.

The emerging roles of plant hormones and nanoparticles in modulating various abiotic stresses have been extensively evaluated (Ghassemi-Golezani and Abdoli, 2021; Singh *et al*., 2021 b). Salicylic acid (SA) as a naturally phenolic hormone has effectual roles in numerous metabolic processes and regulates photosynthesis and antioxidant activities, redox and osmotic hemostasis, ionic uptake and secondary metabolite synthesis in plants exposed to salinity (Abdoli and Ghassemi-Golezani, 2021; Hussain *et al*., 2021). Salicylic acid can reverse the ethylene-induced detrimental impacts via modulating the transcription of *ACS*, *NHX*, *sos1*, *HKT1* and *HKT2* genes, and improving antioxidants capacity, leading to an increase in shoot and root growth, leaves per plant, leaf area and plant productivity (Rao *et al*., 2021). Simultaneous application of SA and nanoparticles can be more effective in augmenting the ameliorative effects of SA under salt stress (Mozafari *et al*., 2018; Ghassemi-Golezani and Abdoli, 2021). Nanoparticles (NPs) rapidly penetrate the plant cell due to their small size and high solubility, thereby compensating the nutrient deficiencies. Through phloem vessels and plasmodesmata, foliar applied nanoparticles can be transferred into the cells (Knoblauch and Oparka, 2012). The bond between carrier proteins and nanoparticles facilitates the entry of nanoparticles into the cells through ion channels, aquaporin, and endocytosis (Nair *et al.,* 2010). Since the uptake of most micronutrients is reduced under salinity, supplying nano-forms of these elements not only reduces nutritional imbalance, but also helps the plants to cope with stress through various physiological and metabolic changes. For instance, silica NPs boost salt tolerance by regulating ion homeostasis, osmotic adjustment and chlorophyll content, which recover plant growth and productivity (Alsaeedi *et al*., 2019). Iron oxide NPs may have critical roles in different biochemical synthesis, antioxidant activity and genes expression (Moradbeygi *et al*., 2020). The Fe_2O_3-NPs induced salt tolerance in *Moldavian balm* plants was due to augmenting DPPH radical scavenging activity, biochemical compounds accumulation and stimulating expression of genes involved in the bio-synthesis pathway of important phenolic acids such as rosmarinic acid (Moradbeygi *et al*., 2020). Recent investigations indicated that foliar spray of Fe_2O_3-NPs on plants under salt stress notably increased chlorophyll concentration, carbohydrate content (i.e., sugars), and enzymatic defense capacity. Moreover, it decreased lipid peroxidation and ROS generation (Singh *et al*., 2021 a). According to Dola *et al*. (2022) application of 200 ppm iron-oxide nanoparticles resulted in an improvement of plant growth, relative water content chlorophyll content, 100-seed weight, seed yield, and protein and oil contents of soybean. Adding Fe_2O_3-NPs to the soil enhanced leaf area, leaf number per plant, shoot length, and shoot and root weights of tomatoes (El-Desouky *et al*., 2021). Improving plant growth by iron oxide nanoparticles was also observed by several studies on wheat (Rizwan *et al*., 2019; Manzoor *et al*., 2021; El-Saber *et al*., 2021).

Ajowan (*Trachyspermum ammi* L.) is a medicinal plant belonging to the Apiaceae family.It is well known for its essential oil (up to 5%), particularly in seeds (Minija and Thoppil, 2002). Due to numerous pharmacological properties of ajowan essential oil including stimulant, antiseptic, anesthetic, antimicrobial, antiviral, antiulcer, antihypertensive, antitussive, antihyperlipidemic and bronchodilatory, this plant is widely employed (Bhadra, 2020). In our previous reports (Abdoli *et al*., 2020; Ghassemi-Golezani and Abdoli, 2021) the mechanisms of improving salt tolerance in ajowan plants by salicylic acid and iron oxide nanoparticles were discussed in details. In addition to the reported results, this research aimed at evaluating the growth responses of ajowan to these treatments focusing on root and shoot growth, and yield-related traits under salt stress.

2. Materials and Methods

Experimental conditions and treatments

Two pot experiments with a factorial arrangement based on a randomized complete block design in three replicates were set up in a greenhouse at the University of Tabriz, Iran, to investigate the effects of individual and simultaneous application of SA (1 mM) and Fe_2O_3-NPs (3 mM) on sodium, potassium and calcium contents, root and shoot growth, seed filling and yield parameters of salt-stressed (0, 4, 8 and 12 dS m^{-1} NaCl; as non-saline and low, moderate and high salinities, respectively) ajowan plants. The salinity (Nikpour-Rashidabad *et al.,* 2022) and foliar spray (Hussain *et al.,* 2019; Ghassemi-Golezani and Farhadi, 2022) levels were selected according to previous reports. The average temperatures of day and night, relative humidity and light intensity in the greenhouse were 29°C, 25°C, 35-40%, and 141 Wm^{-2} (about 780 µmol $m^{-2}s^{-1}$), respectively.

This research was performed with 52 pots (48 pots for sowing and 4 unsown pots for checking the water status). Ajowan seeds (30 seeds per pot) were sown in each pot in 1 cm depth of a mixture of perlite and cocopeat to keep long-term moisture in the substrate. The tested salt solutions were added to the substrate of the pots up to 100% field capacity (FC). The emerged seedlings were reduced to keep 10 plants per pot. The water loss from the pots was compensated by tap water or Hoagland solution (EC=1.3 dS m^{-1}, pH=6.7-7.2) up to 100% FC. Toprevent excess increment of EC in the substrate due to Hoagland addition, the perlite + cocopeat within all pots were washed slowly every 30 days by pouring water into the pots and draining from the lower holes of the pots. The EC of draining water was measured frequently and when the pouring and draining waters showed similar ECs, washing was stopped and then re-treated with salt solutions. The SA, Fe_2O_3-NPs and tap water were sprayed on plants at two different stages (7 leaves and flowering), by a two-liter manual sprayer.

Estimation of Na^+, K^+ and Ca^{2+} contents

The sodium, potassium and calcium contents in plant tissues were determined by a flame photometer (Corning flame photometer, 410). The samples of ajowan plants were reduced to dry ashes in an electric furnace at 500℃ for 7 h, and the carbon-free residue was then dissolved in 1 N HCl. The Na^+, K^+ and Ca^{2+} contents were determined as milligrams per gram dry weight.

Measurement of root and shoot parameters

Two plants from each pot were removed at maturity. The roots were cut from the crown and thoroughly washed and air-dried. Then, the root and shoot lengths, root diameter, branches per plant and leaves per plant were recorded. Subsequently, the samples of roots and shoots were separately dried at 75℃ for 48 h and weighed.

Seed filling

During seed filling in 2018, two plants from each pot were harvested at 10 days intervals, beginning 20 days after flowering and then seeds were removed from the plants and weighed at five stages.

Yield parameters

The seeds of two plants from each pot were separated and then the number of umbels per plant, seeds per umbel, seeds per plant, 1000-seed weight and seed yield were determined. The harvest index was calculated as:

$$\text{Harvest index} = (\text{Seed yield/shoot + seed mass}) \times 100$$

Statistical analysis

All collected data in this study were subjected to a two-way analysis of variance (ANOVA) using MSTAT-C, and means were compared by Duncan' s multiple range test at $p \leq 0.05$. The mean data were presented as means ± standard error. Pearson correlation coefficient was used to analyze the relations between morphological and yield-related traits of ajowan plants, using SPSS 16.

3. Results

The Na^+, K^+ and Ca^{2+} contents

The Na^+, K^+ and Ca^{2+} contents and K^+/Na^+ and Ca^{2+}/Na^+ ratios were significantly affected by salt stress and foliar treatments ($p \leq 0.01$). The K^+ and Ca^{2+} contents and K^+/Na^+ and Ca^{2+}/Na^+ ratios were decreased, while the Na^+ content was increased by the increment of salt toxicity. The 4 dS m^{-1} NaCl had no significant impact on Ca^{2+} content. Foliar treatments, particularly SA + Fe_2O_3-NPs, reduced Na^+ content and enhanced K^+ and Ca^{2+} contents and K^+/Na^+ and Ca^{2+}/Na^+ ratios. Differences between SA and SA + Fe_2O_3-NPs treatments in Na^+ and Ca^{2+}

contents and Ca^{2+}/Na^{+} ratio were not statistically significant. All of these parameters (except K^{+}/Na^{+} ratio) were similarly affected by SA and Fe_2O_3-NPs treatments (Table 1).

Root growth

Significant interaction of salt stress and foliar treatments were observed for root growth parameters ($p \leq 0.01$). The length, weight and diameter of ajowan roots were decreased as salinity increased. No differences among foliar treatments in root parameters were recorded under low salinity. Nonetheless, foliar sprays significantly promoted root growth of plants at different saline conditions, especially under moderate and high salinities. The SA and SA + Fe_2O_3-NPs treatments were the superior treatments in improving root growth (Table 2). In most cases, the differences betweenhormonal and nutritional treatments were not statistically significant (Table 2).

Shoot parameters

A significant interaction of salinity and foliar applications was observed for shoot mass and length, branches and leaves per plant (Table 3). Increasing salinity significantly decreased shoot parameters. The shoot length of treated and untreated plants was not significantly varied under non-saline conditions. However, foliar treatments significantly increased shoot length under all salinity levels. The shoot mass, and branches and leaves per plant were enhanced by different foliar treatments, especially by SA + Fe_2O_3-NPs, under saline and non-saline conditions. The differences among SA, Fe_2O_3-NPs and SA + Fe_2O_3-NPs treatments in shoot mass under 4 dS m^{-1}NaCl, in shoot length under 4 and 8 dS m^{-1} NaCl and in branches per plant under 8 dS m^{-1} NaCl were not significant (Table 3).

Seed filling

The dry weight of ajowan seeds was gradually enhanced with seed development up to about 50 days after flowering and afterwards no significant changes occurred. Seed dry weight was not significantly affected by low salinity (4 dS m^{-1} NaCl), but further increment of salt stress, particularly high salinity, caused a significant decrease in the dry weight of ajowan seeds at later stages of seed development under all foliar treatments. Application of SA, Fe_2O_3-NPs individually and in combination form increased seed weight under moderate and high salinities. This improvement was mainly due to increasing seed filling rate rather than seed filling duration (Fig. 1).

Yield components

The interaction of salinity and foliar treatments was significant for umbels per plant, seeds per plant, 1000-seed weight, seed yield and harvest index (Table 4). Seeds per umbel were only affected by salt stress. Low salinity had no significant effect on seeds per umbel. However, further increment in salinity

Table 1 - The Na^{+}, K^{+} and Ca_2^{+} contents (mg g^{-1} dry weight) of ajowan plants affected by foliar treatments under saline and non-saline conditions

Treatments	Na^{+}	K^{+}	Ca^{2+}	K^{+}/Na^{+}	Ca^{2+}/Na^{+}
Salinity conditions					
Non-Saline	9.29 ± 0.45 d	45.17 ± 1.8 a	13.94 ± 0.24 a	5.05 ± 0.38 a	1.53 ± 0.06 a
4 dS m^{-1} NaCl	15.14 ± 1.17 c	40.86 ± 1.8 b	13.19 ± 0.43 a	2.86 ± 0.23 b	0.92 ± 0.08 b
8 dS m^{-1} NaCl	27.57 ± 0.99 b	30.72 ± 1.3 c	10.63 ± 0.55 b	1.15 ± 0.08 c	0.40 ± 0.03 c
12 dS m^{-1} NaCl	32.35 ± 1.51 a	28.01 ± 1.2 d	7.82 ± 0.72 c	0.90 ± 0.07 d	0.25 ± 0.03 d
F test	479.64 **	220.88 **	60.57 **	679.22 **	369.80 **
Foliar treatment					
Water	26.39 ± 3.52 a	28.36 ± 1.74 c	9.91 ± 1.19 c	1.53 ± 0.33 d	0.57 ± 0.14 c
SA	19.53 ± 2.72 b	37.97 ± 2.49 b	11.86 ± 0.64 ab	2.82 ± 0.61 b	0.86 ± 0.17 ab
Fe_2O_3-NPs	19.30 ± 2.47 b	37.16 ± 2.22 b	11.23 ± 0.81 b	2.56 ± 0.46 c	0.78 ± 0.15 b
SA+Fe_2O_3-NPs	19.14 ± 2.60 b	41.28 ± 2.29 a	12.58 ± 0.58 a	3.04 ± 0.63 a	0.89 ± 0.16 a
F test	52.44 **	101.29 **	10.15 **	82.99 **	22.85 **

Different letters in each column indicate significant differences at p≤0.05; ** = significant at p≤0.01.
Fe_2O_3-NPs= Iron-oxide nanoparticles; SA= Salicylic acid.

Table 2 - Combined analysis of variance of the data for root growth parameters of ajowan affected by foliar treatments under saline and non-saline conditions in 2018 and 2019

Salinity	Foliar treatments	Root mass (g)	Root length (cm)	Root diameter (mm)
Non-Saline	Water	1.88 ± 0.02 abc	32.98 ± 0.05 abc	2.63 ± 0.17 a
	SA	1.97 ± 0.04 a	34.28 ± 0.6 ab	2.51 ± 0.11 a
	Fe_2O_3-NPs	1.94 ± 0.05 ab	34.48 ± 0.5 a	2.37 ± 0.11 ab
	SA+ Fe_2O_3-NPs	1.99 ± 0.05 a	34.23 ± 0.4 ab	2.57 ± 0.12a
4 dS m-1 NaCl	Water	1.74 ± 0.05 ef	28.95 ± 0.6 d	2.41 ± 0.08 ab
	SA	1.85 ± 0.03 bcd	32.95 ± 0.5 abc	2.43 ± 0.08 ab
	Fe_2O_3-NPs	1.83 ± 0.04 cde	31.83 ± 0.8 c	2.38 ± 0.7 ab
	SA+ Fe_2O_3-NPs	1.90 ± 0.04 abc	32.20 ± 0.8 bc	2.48 ± 0.08 a
8 dS m-1 NaCl	Water	1.23 ± 0.05 h	17.12 ± 0.7 hi	1.88 ± 0.06 def
	SA	1.76 ± 0.02 de	24.02 ± 0.8 ef	2.13 ± 0.06 bcd
	Fe_2O_3-NPs	1.64 ± 0.03 f	22.47 ± 0.8 f	2.31 ± 0.05 abc
	SA+ Fe_2O_3-NPs	1.80 ± 0.03 cde	25.75 ± 1.0 e	2.32 ± 0.10 abc
12 dS m-1 NaCl	Water	0.83 ± 0.06 i	14.37 ± 0.6 j	1.62 ± 0.06 f
	SA	1.50 ± 0.04 g	19.03 ± 0.4 gh	2.05 ± 0.07 cd
	Fe_2O_3-NPs	1.21 ± 0.06 h	16.48 ± 0.5 i	1.72 ± 0.09 ef
	SA+ Fe_2O_3-NPs	1.48 ± 0.02 g	19.90 ± 0.6 g	2.00 ± 0.06 de
Source of variation				
Year (Y)		NS	NS	NS
Salinity (S)		**	**	**
Foliar treatments (F)		**	**	*
Y x S		*	*	NS
Y x F		NS	NS	NS
S x F		**	**	*
Y x SxF		NS	NS	NS
F test		17.14**	4.39**	2.36*

Different letters in each column indicate significant differences at p≤0.05;
NS, *, **= No significant and significant at p≤0.05 and p≤0.01, respectively.
Fe_2O_3-NPs= Iron-oxide nanoparticles; SA= Salicylic acid.

considerably reduced this parameter. The plants grown under high salinity had the lowest seeds per umbel compared to unstressed plants (Fig. 2).

Rising salinity significantly reduced the other yield parameters and harvest index. Foliar treatments had no significant effects on 1000-seed weight under non-saline conditionsand on umbels per plant, seeds per plant and harvest index under non-saline and low salinity. However, hormonal and nutritional treatments enhanced these parameters under saline conditions. This improvement was more evident under moderate and high salinities. In these levels of salinities, the SA and SA + Fe_2O_3-NPs were the best treatments for improving yield parameters, followed by Fe_2O_3-NPs (Table 4).

Correlations

All morphological and yield parameters of ajowan had asignificant positive correlation with each other (Table 5). Root and shoot masses and lengths were highly related toeach other and with leaves per plant ($r \geq 0.85^{**}$). The root and shoot parameters as well as yield components and harvest index were positively and significantly correlated with seed yield per plant. However, the highest relations with seed yield were recorded for shoot mass and seeds per plant, followed by shoot and root lengths, leaves per plant and 1000-seed weight (Table 5).

4. Discussion and Conclusions

The Na^+ toxicity negatively influenced different aspects of plant growth such as root and shoot parameters, resulting in less seed production. The salt-treatedajowan plants responded to foliar applications, particularly to SA and SA + Fe_2O_3-NPs treatments, as demonstrated by higher K^+ and Ca^{2+}

Table 3 - Combined analysis of variance of the data for shoot mass, shoot length, brunches and leaves of ajowan plants affected by foliar treatments under saline and non-saline conditions in 2018 and 2019

Salinity	Foliar treatments	Shoot mass (g)	Shoot length (cm)	Branches per plant	Leaves per plant
Non-Saline	Water	15.48 ± 0.18 b	99.87 ± 0.72 abc	11.85 ± 0.74 d	58.47 ± 1.10 ab
	SA	16.08 ± 0.20 a	101.1 ± 0.78 ab	13.82 ± 0.29 ab	57.33 ± 0.88 b
	Fe_2O_3-NPs	15.97 ± 0.22 a	99.93 ± 0.70 abc	12.33 ± 0.48 cd	59.10 ± 0.53 ab
	SA+Fe_2O_3-NPs	16.17 ± 0.22 a	101.7 ± 0.96 a	14.62 ± 0.44 a	60.78 ± 0.91 a
4 dS m^{-1} NaCl	Water	14.22 ± 0.15 d	95.22 ± 0.70 e	10.50 ± 0.36 e	45.00 ± 0.86 de
	SA	14.73 ± 0.22 c	96.93 ± 0.99 de	13.40 ± 0.43 bc	51.33 ± 0.95 c
	Fe_2O_3-NPs	14.70 ± 0.20 c	99.03 ± 0.64 bcd	12.40 ± 0.34 cd	47.10 ± 0.80 d
	SA+Fe_2O_3-NPs	14.90 ± 0.21 c	97.78 ± 0.61 cd	14.17 ± 0.37 ab	50.77 ± 0.68 c
8 dS m^{-1} NaCl	Water	8.63 ± 0.27 g	68.67 ± 0.71 gh	8.85 ± 0.31 fg	31.67 ± 0.80 i
	SA	12.42 ± 0.27 e	79.22 ± 0.67 f	11.72 ± 0.37 d	41.57 ± 0.69 fg
	Fe_2O_3-NPs	11.35 ± 0.34 f	78.82 ± 0.66 f	11.75 ± 0.45 d	39.47 ± 0.46 g
	SA+Fe_2O_3-NPs	12.49 ± 0.25 e	80.87 ± 0.68 f	11.67 ± 0.37 d	42.67 ± 0.56 ef
12 dS m^{-1} NaCl	Water	4.52 ± 0.27 j	61.23 ± 0.61 i	8.17 ± 0.42 g	24.00 ± 0.89 j
	SA	7.54 ± 0.12 h	69.03 ± 0.79 g	9.05 ± 0.28 fg	35.33 ± 0.84 h
	Fe_2O_3-NPs	6.25 ± 0.23 i	66.27 ± 0.60 h	8.48 ± 0.38 g	30.70 ± 0.63 i
	SA+Fe_2O_3-NPs	7.73 ± 0.09 h	68.80 ± 0.98 g	9.72 ± 0.33 ef	34.67 ± 0.92 h
Source of variation					
Year (Y)		NS	NS	*	ns
Salinity (S)		**	**	**	**
Foliar treatments (F)		**	**	**	**
Y × S		NS	NS	*	NS
Y × F		NS	NS	NS	NS
S × F		**	**	**	**
Y × S × F		NS	NS	NS	NS
F test		21.99**	11.64**	3.05**	7.81**

Different letters in each column indicate significant differences at p≤0.05;
NS, *, **= No significant and significant at p≤0.05 and p≤0.01, respectively.
Fe_2O_3-NPs= Iron-oxide nanoparticles; SA= Salicylic acid.

contents in plant tissues, root and shoot growth and yield-related traits. A high concentration of Na^+ ions in the substrate led to an increase in Na^+ uptake and accumulation in plant tissues and a decline in K^+ and Ca^{2+} uptake, and K^+/Na^+ and Ca^{2+}/Na^+ ratios (Table 1). This imbalance in the nutrient status of tissues might be due to an injury in the cell membrane and specific ion channels (Jayakannan *et al*., 2013). However, the reduction of Na^+ accumulation by foliar spray, particularly by SA + Fe_2O_3-NPs, enhanced the uptake of essential nutrients. This improvement could be related to the activation of H^+-ATPase and H^+-PPase pumps by SA and Fe_2O_3-NPs treatments that induces Na^+ secretion in vacuoles (Ghassemi-Golezani and Abdoli, 2021) and helps plants cope withsalt toxicity.Our results suggest that the enhancement of root growth by these treatments (Table 2) is effective in improving nutrient availability to the plants.The manganese-iron nanoparticles have been reported to increase the efflux of H^+ and influx of K^+, leading to high K^+/Na^+ ratio (Wang *et al.,* 2022).

The roots play an imperative part in plant establishment in the soil and water and nutrient absorptions. A comprehensive understanding of root response to the foliar spray of SA and Fe_2O_3-NPs can more likely provide useful information for improving

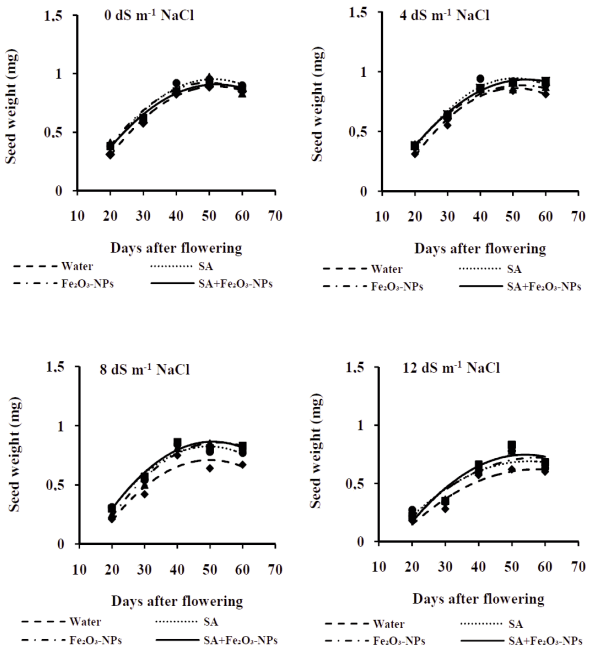

Fig. 1 - Changes in mean seed weight during seed development in response to salinity and foliar treatments. Fe_2O_3-NPs= Iron oxide nanoparticles, SA= Salicylic acid.

crop productivity in saline soils. The length, mass and diameter of roots in salt-stressed plants, especially under moderate and high salinities, were limited due to nutritional (Table 1) and hormonal imbalances (Zhang *et al*., 2018), that limit cell elongation and division (Yang *et al*., 2019). The GiA Roots analysis alsorevealed a decrease in the penetration and distribution of plant roots under different levels of salinities, particularly under high saline conditions (Ghassemi-Golezani and Abdoli, 2022 b). These negative impacts of salinity on root growth were relieved by foliar treatments of SA and Fe_2O_3-NPs (Table 2). The cross-talk of SA with auxins, cytokinins and gibberellins (Shakirova *et al*., 2003; Agami and Mohamed, 2013; Miura *et al*., 2013) can potentially promote cell elongation and division. In addition, the expression of auxin biosynthesis genes might be regulated by Fe status of cells (Sun *et al.,* 2017). Iron-NPs may enhance root growth by inducing OH radical generation and demolition of cell wall polysaccharides (Kim *et al*., 2014). Stimulation of SA synthesis in plants by Fe_2O_3-NPs related treatments (Abdoli *et al*., 2020) is also effective in improving plant growth.

Reduction in shoot mass and length, and branches and leaves per plant due to salinity (Table 3) is related to Na^+ toxicity and competition of plants for nutrients (Table 1) and water (Abdoli *et al*., 2020). This is a mechanism for minimizing energy losses and maintaining plant survival chance under stress conditions. It has been confirmed that salt stress leads to growth reduction, which is more pronounced in leaf area (Acosta-Motos *et al*., 2015), branches and leaves per plant and shoot length and mass (Table 3). Foliar treatments promoted shoot growth by enhancing root growth (Table 2), and improving K^+ and Ca^{2+} contents in plant tissues bylimiting Na^+ absorption by the plants (Table 1). The effectiveness of salicylic acid inpromoting cell division and enlargement is also supported by a previous report on wheat plants (Agami and Mohamed, 2013). Inhibition of ethylene synthesis by SA (Khan *et al*., 2014) can enhance the plant growth duration. A decline in abscisic acid contentdue to iron nanoparticlesmay also promote growth and retard the senescence of plants (Rui *et al*., 2016). Moreover, iron nanoparticlesareinvolved in protein synthesis and enzymes activation, which can promote the plant growth and reduce the senescence especially under stressful conditions (Sheykhbaglou *et al.,* 2018). Wang *et al.* (2022) suggested that cytokinin level, *SCFTIR1/AFB-AUX/IAA* signaling pathway, ATP synthesis, cell elongation and plant biomass could be enhanced by iron nanoparticles.

Decreasing root- and shoot-related traits (Tables 2 and 3) due to salt stress reduced yield parameters including seed filling rate (Fig. 1), seeds per plant, 1000-seed weight and seed yield (Table 4; Fig. 2). These reductions are most likely attributed to the enhanced vegetative and reduced reproductive periods under salinity (Ghassemi-Golezani and Farhangi-Abriz, 2021). Retarding flowering due to salinity reduced umbels and seeds per plant, 1000-seed weight, and consequently seed yield (Table 4). The reduction in photosynthetic efficiency (Ghassemi-Golezani *et al*., 2021) and allocation of assimilates to the seeds (Kafi *et al*., 2013) might be the main reasons for yield losses under moderate and high salinities. The SA + Fe_2O_3-NPs treatment alleviated these detrimental impacts of salinity on plant productivity through the improvement of nutrient availability (Table 1), root (Table 2) and shoot (Table 3) growth, photosynthetic potential (Ghassemi-Golezani and Farhadi, 2022) and stimulation of flower-inducing factor (Hayat *et al*., 2007). In a study on salt-stressed pennyroyal plants, the SA application enhanced Rubisco activity, a

Table 4 - Combined analysis of variance of the data for yield parameters of ajowan affected by salinity and foliar treatments in 2018 and 2019

Salinity	Foliar treatments	Umbels per plant	Seeds per plant	1000-seed weight (mg)	Seed yield (g plant^{-1})	Harvest index (%)
Non-Saline	Water	50.67 ± 0.42 a	6367.9 ± 56.7 a	865.7 ± 3.7 ab	5.51 ± 0.07 b	35.63 ± 0.06 a
	SA	51.00 ± 1.24 a	6498.5 ± 110.0 a	888.3 ± 6.0 a	5.77 ± 0.07 a	35.88 ± 0.19 a
	Fe_2O_3-NPs	52.00 ± 0.36 a	6577.6 ± 30.5 a	870.0 ± 6.8 ab	5.72 ± 0.07 a	35.84 ± 0.16 a
	SA + Fe_2O_3-NPs	51.18 ± 0.76 a	6527.8 ± 119.1 a	882.2 ± 7.0 a	5.75 ± 0.07 a	35.59 ± 0.14 a
4 dS m^{-1} NaCl	Water	45.68 ± 0.92 b	5760.1 ± 47.0 b	829.3 ± 9.2 cd	4.78 ± 0.08 d	33.60 ± 0.28 b
	SA	45.22 ± 0.23 b	5751.6 ± 89.0 b	881.7 ± 4.7 a	5.07 ± 0.07 c	34.43 ± 0.17 b
	Fe_2O_3-NPs	45.83 ± 0.54 b	5874.3 ± 71.0 b	854.3 ± 8.2 bc	5.02 ± 0.07 c	34.14 ± 0.17 b
	SA + Fe_2O_3-NPs	46.83 ± 0.31 b	5919.4 ± 85.5 b	866.0 ± 3.6 ab	5.12 ± 0.06 c	34.40 ± 0.24 b
8 dS m^{-1} NaCl	Water	33.72 ± 0.77 e	3953.6 ± 144.3 e	669.3 ± 9.6 f	2.64 ± 0.06 g	30.63 ± 0.45 d
	SA	39.35 ± 0.83 c	4789.9 ± 97.4 c	827.5 ± 7.0 d	3.96 ± 0.07 e	31.92 ± 0.31 c
	Fe_2O_3-NPs	36.65 ± 0.78 d	4437.6 ± 106.2 d	780.3 ± 6.9 e	3.46 ± 0.06 f	30.55 ± 0.53 d
	SA + Fe_2O_3-NPs	39.78 ± 1.00 c	4852.7 ± 120.6 c	836.2 ± 12.8 cd	4.05 ± 0.08 e	32.45 ± 0.30 c
12 dS m^{-1} NaCl	Water	19.57 ± 0.72 g	1946.3 ± 108.7 h	605.0 ± 2.2 h	1.18 ± 0.07 j	26.10 ± 0.51 f
	SA	31.75 ± 0.37 e	3326.2 ± 117.0 f	687.7 ± 12.8 f	2.27 ± 0.03 h	30.19 ± 0.18 d
	Fe_2O_3-NPs	27.18 ± 1.04 f	2820.9 ± 87.3 g	630.0 ± 2.6 g	1.78 ± 0.05 i	28.47 ± 0.30 e
	SA + Fe_2O_3-NPs	33.32 ± 0.44 e	3471.7 ± 102.6 f	686.7 ± 8.8 f	2.38 ± 0.05 h	30.79 ± 0.42 d
Source of variation						
Year (Y)		NS	NS	NS	NS	NS
Salinity (S)		**	**	**	**	**
Foliar treatments (F)		**	**	**	**	**
Y × S		NS	NS	NS	**	NS
Y × F		NS	NS	NS	NS	NS
S × F		**	**	**	**	**
Y × S × F		NS	NS	NS	NS	NS
F test		13.19**	12.73**	12.92**	32.35**	9.78**

Different letters in each column indicate significant differences at p≤0.05;
NS, **= No significant and significant at p≤0.01, respectively.
Fe_2O_3-NPs= Iron-oxide nanoparticles; SA= Salicylic acid.

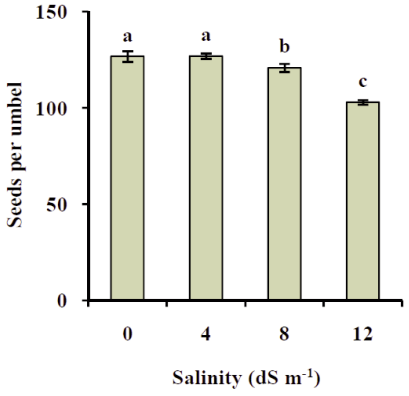

Fig. 2 - Changes in seeds per umbel of ajowan in response to salinity. The data represents the average of three replicates in two years ± standard errors. Different letters indicate significant differences at *p*≤0.05.

critical enzyme in photosynthetic machinery.The enhanced seed-filling rate (Fig. 1) by simultaneous application of SA and Fe_2O_3-NPs under moderate and high salinities resulted in the production of larger seeds (Table 4). The high correlation of root and shoot parameters, seeds per plant and 1000-seed weight with seed yield (Table 5) suggests that improving these traits by breeding or foliar treatments can potentially increase crop productivity under normal and stressful conditions.Our results indicated that foliar spray of SA and SA + Fe_2O_3-NPs often similarly improves salt tolerance and seed yield of ajowan, so application of SA and/or SA + Fe_2O_3-NPs treatments can be cost-effective in reducing salinity-induced losses in large-scale production systems.

Salinity remarkably limited the root and shoot

Table 5 - Correlations of morphological and yield parameters of ajowan with each other

Parameters	Root mass	Root length	Shoot mass	Shoot length	Branches per plant	Leaves per plant	Seeds per plant	1000 seed weight	Seed yield	Harvest index
Root mass	1									
Root length	0.86 **	1								
Shoot mass	0.91 **	0.94 **	1							
Shoot length	0.85 **	0.96 **	0.96 **	1						
Branches per plant	0.77 **	0.80 **	0.82 **	0.81 **	1					
Leaves per plant	0.87 **	0.94 **	0.93 **	0.93 **	0.79 **	1				
Seeds per plant	0.90 **	0.94 **	0.99 **	0.95 **	0.79 **	0.94 **	1			
1000-seed weight	0.90 *	0.91 **	0.95 **	0.92 **	0.83 **	0.89 **	0.92 **	1		
Seed yield	0.90 *	0.96 **	0.99 **	0.97 **	0.82 **	0.95 **	0.99 **	0.95 **	1	
Harvest index	0.86 **	0.92 **	0.92 **	0.92 **	0.75 **	0.94 **	0.94 **	0.91 **	0.94 **	1

** Significant at p≤0.01.

growth of ajowan, leading to lower seed yield.Negative impacts of salt stress on plant growth and productivitycould be considerably alleviated by exogenous salicylic acid and Fe_2O_3 nanoparticles, particularly in combined form. These beneficial effects were more pronounced under severe salinity. The ameliorative effects of SA and SA+ Fe_2O_3-NPs on seed yield of salt-subjected ajowan plants were mostly related to enhancing root and shoot growth, seeds per plant, seed filling rate, and 1000-seed weight. Future works may reveal other beneficial effects of different hormones and/or nanoparticles on crops under various environmental conditions.

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Field evaluation of biostimulants on growth, flowering, yield, and quality of snap beans in subtropical environment

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Key words: Chitosan, potassium silicate, 6-benzylaminopurine, snap bean, triacontanol.

Abstract: The cultivation of snap beans (*Phaseolus vulgaris* L.) in subtropical regions faces environmental challenges leading to potential declines in yield. This study explores the efficacy of biostimulants as a solution, specifically investigating spraying treatments with 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), and potassium silicate (KSi) on the snap bean cv. Paulista. Over two growing seasons with late sowing and elevated summer temperatures, the research assesses growth, flowering, yield, and quality. Notably, 5 ppm TRIA demonstrates the most significant impact on plant growth and leaf nutrient content. Treatments with 40 ppm 6-BA, 5 ppm TRIA, or 200 ppm KSi exhibit notable effects on inflorescence flower count and flowers per plant. These treatments prove most effective for crucial green pod yield measures, including the number and weight of marketable pods. Moreover, 40 ppm 6-BA or 5 ppm TRIA significantly enhances pod characteristics, such as length, diameter, and weight, consistently improving over both seasons. Particularly, 5 ppm TRIA outperforms in enhancing the chemical quality of pods throughout the study. Overall, the findings suggest that the application of 5 ppm TRIA offers the most favorable enhancements for the growth, flowering, productivity, and quality of snap bean plants in subtropical field conditions.

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1. Introduction

The global population is now and will continue to exert increased pressure on the need for food. Hence, it is essential for farmers to annually increase food production with the existing resources to fulfill this demand. Common bean (*Phaseolus vulgaris* L.) is a common vegetable that includes both snap and dry beans (Lin *et al*., 2008). In Egypt, farmers dedicate 27363 hectares to green bean cultivation, yielding 284299 tons annually (FAOSTAT, 2021).However, snap beans have a notable suscepti-

bility to high summer temperatures, particularly when subjected to delayed planting, like in April and May under Egyptian field conditions (El-Bassiony *et al.*, 2012). While it has been stated that the optimal temperature for bean plants is 23°C (Dickson and Boettger, 1984). Omae *et al.* (2006) observed that the occurrence of high summer temperatures (26°C Min and 30°C Max.) during the initiation of the blooming stage had an adverse impact on the quantity and the weight of pods. Along with this, climate models predict a 50% decrease in global cultivated area by 2050 due to global warming (Rippke *et al*., 2016; Rama Rao *et al*., 2022).

One potential approach to enhancing snap bean production is through the breeding of new cultivars. However, it is important to note that this process often takes a significant amount of time and may provide limited results (Xiong *et al*., 2022). Biostimulants provide a compelling alternative in the context of degraded agricultural regions and the risks associated with climate change. In recent times, there has been increased research focusin the utilization of biostimulantsin the form of plant growth regulators (e.g., 6-benzylaminopurine; 6-BA as a synthetic cytokinin, and triacontanol; TRIA), chitosan (Ch), and trace elements (e.g., silicon; Si) that have been found effective in improving plant productivity (Du Jardin, 2015; Yaghubi *et al*., 2019; Islam and Mohammad, 2020; Hassan *et al*., 2021; Stasińska-Jakubas and Hawrylak-Nowak, 2022). Although cytokinins (CKs) have vital function in controlling plant development, they have also been shown to confer other benefits, such as improving photosynthetic rates, photosynthetic pigments, and nutrient uptake (Aremu *et al*., 2020; Li *et al*., 2021). In a study conducted by Mostafa and Brengi (2018), it was shown that the application of 6-BA solution on okra leaves resulted in improved yield and chemical composition. Furthermore, Yang *et al*. (2016) illustrated that the treatment with 6-BA resulted in an improvement in several aspects of wheat grain development, including wheat grain filling and endosperm cell division under heated growth conditions. It is a widely recognized that TRIA is plant growth regulator (Islam and Mohammad, 2020). Triacontanol is a saturated alcohol initially discovered in alfalfa (Ries *et al*., 1977) and is found naturally as a wax coating on a variety of plant species (Islam and Mohammad, 2020). In addition to its function in eliciting responses to stresses, TRIA is participated in plant growth, production, and vital physiological processes (Faiz *et al*., 2024). In this manner, Waqas *et al*. (2016) showed that both normal growth and heat stress conditions, TRIA treatment of mung bean plants resulted in improved plant growth, leaf chlorophyll content, nutrients, and protein content. Chitosan is a naturally carbohydrate polymer that has been produced from chitin, a substance found in the shells of crustaceans (Hidangmayum *et al*., 2019). It is non-toxicand biocompatible, making it potentially useful in agriculture and biotechnology (Stasińska-Jakubas and Hawrylak-Nowak, 2022). Basically, Ch improves physiological responses and reduces the negative effects of abiotic stressors through the secondary messengers (Hidangmayum *et al*., 2019). Therefore, Ch is thought to be a viable exogenous addition for increasing crop production and overcoming abiotic stress (Stasińska-Jakubas and Hawrylak-Nowak, 2022). Apart from this, Ch also enhanced the productivity of many crops such as tomatoes (El-Tantawy, 2009), cowpea (Farouk and Amany, 2012) and cucumber (Ali *et al*., 2020). Generally, silicon (Si)ranks among the most abundant elements found in soil (Souri *et al*., 2021). Recently, the connections between Si and various biological processes in multiple crops were clarified, and silicon was recognized as one of the vital nutrients required by plants (Zargar *et al*., 2019). Silicon is engaged in many biological activities such as photo synthesis, nutrient uptake, and plant adaptation to stress (Zargar *et al*., 2019; Souri *et al*., 2021). Potassium silicate (KSi) is usually used as biostimulant and a producer of both soluble K and Si (Yaghubi *et al*., 2019). It is well recognized that K is a core element and participates in a vital function in cell division, protein synthesis, the formation of sugars, and plant growth, as well as vital processes such as plant photosynthesis and stomata movement (Ali *et al*., 2021).

Although previous studies have examined the individual impacts of these elicitors on plant growth, a comprehensive investigation into their effects specifically on snap bean plants remains lacking. Moreover, these studies evaluated different parameters and were conducted in different growing environments; consequently, the field evaluation of these biostimulants under a particular subtropical summer conditions are required. Thus, this research was created to test the beneficial impacts of 6-BA, Ch, TRIA, or KSi on the growth, blooming, productivity, and quality of snap bean plants grown in delayed summer cultivation in a subtropical environment.

2. Materials and Methods

Snap bean field conditions

Field trials were undertaken in the Sidi Ghazy Region of Kafr El-Dawar city, located in the Beheira Governorate of Egypt. These experiments were done during the seasons of 2021 and 2022. The geographical coordinates of the study area are around 31°07'N latitude and 30°08' E longitude. The region has an arid climatic condition characterized by an annual precipitation of about 90-110 mm, mostly in the form of ineffectual showers during the winter. Figure 1 presents a summary of the monthly temperatures, measured over the course of two cultivation seasons. The source of this data originates from the Egyptian Ministry of Agriculture and Reclamation of Soils, bulletin of agricultural meteorological data. Samples from the trial soil were subjected to drying and then sifted by a two-mm sieve. These samples were then analyzed using the protocols outlined by Page *et al.* (1982). Experimental soil had a clay texture (22.5% sand, 35.4% silt, and 42.1% clay) with a pH of 8.14, EC value of 1.19 dsm^{-1}, and an organic material level of 1.75%, as an average over the two seasons.

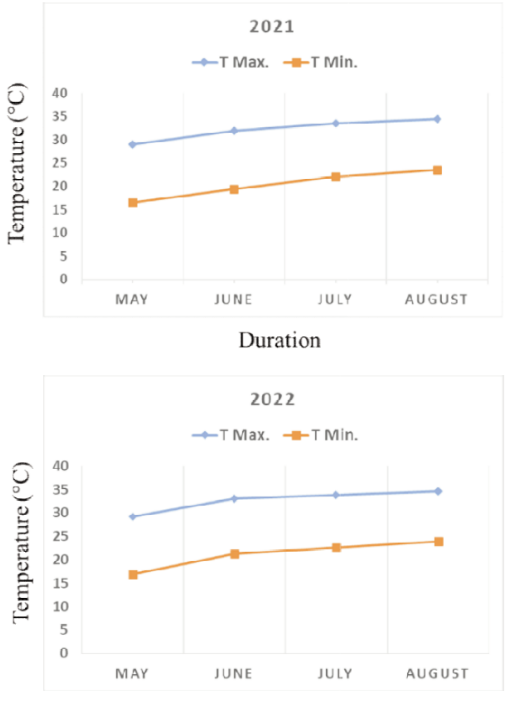

Fig. 1 - Monthly means of maximum (T Max.) and minimum (T Min.) temperature during the two studied seasons (summer 2021 and 2022) (Egyptian Ministry of Agriculture and Reclamation of Soils, bulletin of agricultural meteorological data).

Plant material, experimental design, and treatments

Seeds of the snap bean (*Phaseolus vulgaris* L.) cv. Paulista (Alsafwa company, Egypt) were planted on May 1st and May 2nd in the seasons of 2021 and 2022, respectively. The selection of this cultivar was based on its significant economic worth in both local and foreign markets. The experimental site was plowed and leveled adequately before plots were established in accordance with the experimental layout. The experimental treatments were organized as stated by the Randomized Complete Block Design (RCBD). The trial consisted of 9 treatmentswith3replicates (plots). The seeds were manually cultivated on a single side of the ridge, with a spacing of 10 cm between each seed. The ridge itself had a dimension of 60 cm and 5 m long. Each plot consisted of three ridges, and the total size of each plot was 9 m^2.Two guard ridges were present between each treatment to prevent spray drift.

In this investigation, four biostimulants plus a control were examined, namely 6-benzylaminopurine (6-BA, Sigma-Aldrich, USA) at 20 and 40 ppm, chitosan (Ch, Sigma-Aldrich, USA) at 100 and 200 ppm, triacontanol (TRIA, Sigma-Aldrich, USA) at 2.5 and 5 ppm, potassium silicate (KSi, Sigma-Aldrich, USA) at 100 and 200 ppm, and a control group treated with distilled water. Former studies were employed to identify the suitabledose range of 6-BA (Abouelsaad and Brengi, 2022; Zarea and Karimi, 2023) Ch (Ibrahim and Ramadan, 2015; Stasińska-Jakubas and Hawrylak-Nowak, 2022), TRIA (Islam and Mohammad, 2020), and KSi (Ibrahim *et al.*, 2020). Foliar treatments were employed 3 times, 15 days after seed sowing, followed by further applications every 15 days afterwards. The plants were subjected to a foliar application employing a knapsack sprayer throughout the afternoon until drop-off.

All treatments were provided with an equal total quantity of 50 N, 60 P, and 60 K (kg ha^{-1}) fertilizer for the duration of the season. These fertilizers(surface broadcast application) were given in two equal portions, with the first dosage delivered during the third week after seed planting and the second dose applied during the seventh week. Nitrogen, P, and K were provided as ammonium nitrate, single superphosphate, and potassium sulfate, respectively. All the required practices for cultivating snap beans were properly conducted as required.

Plant growth, flowering, chlorophyll content and mineral analysis

After 50 days of planting, three plants from each

replicate (nine from each treatment) were taken for measuring the height (cm), branch number (plant^{-1}), and number of leaves (plant^{-1}). The relative content of chlorophyll in snap bean were assessed using the Chl meter instrument (SPAD-502) manufactured by Konica Minolta Sensing in Japan. The assessment included measuring the number of inflorescences plant^{-1}, number of flowers in the inflorescence, total number of flowers plant^{-1}, and evaluating the length of the inflorescence (cm) (at 50% flowering according to Schwartz and Langham, 2010) The fresh weight (g) of shoot was measured, and subsequently, the plants were subjected to oven-drying at a temperature of 60°C till their weights stabilized, leading to the determination of the dry weight (g) of the shoot. Plant leaves area (cm^2) was conducted using a mathematical analysis that examined the relationship between the dry weight of leaves (plant^{-1}) and the dry weight and area of 20 discs taken from fresh leaves using a borer with a known diameter. Wallace and Munger (1965) established this relationship and presented it in the following formula:

$$\text{Leaves area (cm}^2\text{)} = \text{leaves dry weight (g)} \times 20 \text{ discs area (cm}^2\text{)} / 20 \text{ discs dry weight (g)}$$

Micro-Kjeldahl was employed to assess Nin snap bean leaves (Sáez-Plaza *et al.*, 2013), but P level was quantified by colorimetric techniques (Watanabe and Olsen, 1965). Zinc, Fe, Mg, and Mn were analyzed in leaves using the atomic absorption spectrophotometer model Perkin Elmer 3100, while K and Ca levels of leaf tissue were quantified by a flame-photometer (CORNING M410) as employed by Munns *et al.* (2010).

Green pod yield and quality

At harvest time (65 days from sowing), observations were recorded for seven characters *viz.,* the number of pods (plant^{-1}), the weight of pods (g plant^{-1}), the number of marketable pods (plant^{-1}), the weight of marketable pods (g plant^{-1}), the mean of pod weight (gm), pod length (cm) and pod diameter (mm). This research considers pods that possess significant quality traits that are important for the export market, such as well-formedness, uniformity, straightness, and absence of flaws, as being considered marketable. Total N (%) was determined in green pods using the micro-Kjeldahl apparatus as defined by Sáez-Plaza *et al.* (2013). Subsequently, total protein (%) was calculated using N% (Mariotti *et al.*, 2008). The detection of vitamin C in the green pods was carried out at a wavelength of 525 nm, utilizing the methodology previously established by Srivastava and Singh (1988). To establish a standard curve, the utilization of ascorbic acid (Analytical Reagent, Solarbio) was employed. The quantification of vitamin C is presented in mg100 g^{-1} FW. The measurements of crude fiber and soluble sugar in pods were conducted using the methodology established by Slavin (1987) and Rady *et al*. (2019), respectively.

Data analysis

The data underwent statistical analysis using a one-way factorial design within the framework of a Randomized Complete Block Design (RCBD). The COSTAT program (CoStat program version 6.311, 2005) was used to conduct a statistical analysis, namely the Duncan's multiple range test, with a significance threshold of $P \leq 0.05$, for the purpose of comparing the means.

3. Results and Discussion

Snap bean growth and chlorophyll contents

Changes in snap bean growth parameters caused by the foliar application of growth elicitors (6-BA, Ch, TRIA, or KSi) are presented in Tables 1 and 2. The application of all treatments boosted plant growth, as clarified by the increases in snap bean height, leaves area, shoot fresh and dry weights. In most instances, the applied treatments also resulted in boosted the number of leaves and branches, although in the case of the treatment with 100 ppm Ch, both seasons' values were similar to the control treatment. Several studies have provided evidence suggesting that the applications of 6-BA, Ch, and KSi have the potential to increase the overall snap bean growth (Werner and Schmülling, 2009; Hidangmayum *et al*., 2019; Yaghubi *et al*., 2019; Gomaa *et al*., 2021). Cytokinins (CKs) are often characterized as hormones that stimulate growth; however, it should be noted that several substances exhibiting CK activity have been discovered to control many features of plant development (Haberer and Kieber, 2002). Cytokinins influence cell multiplication, that in turn influences plant development, and they also promote adventitious buds growth (Kieber and Schaller, 2014). Such substances, including exogenous applications, were used to promote growth in crops and vegetables (Yang *et al*., 2016; Mostafa and Brengi, 2018; El-Areiny *et al*., 2019; Aremu *et al*.,

Table 1 - Plant height, shoot fresh weight, and shoot dry weight of snap beanas affected by 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), and potassium silicate (KSi) in the 2021 and 2022 seasons

Treatment	Plant height (cm)		Shoot fresh weight (g)		Shoot dry weight (g)	
	2021	2022	2021	2022	2021	2022
Control	44.30 e	45.63 d	293.07 d	301.90 d	29.83 d	30.89 e
6-BA (20 ppm)	50.33 bc	52.33 ab	326.02 c	339.03 c	35.64 bc	37.30 cd
6-BA (40 ppm)	52.30 a	53.83 a	364.40 ab	375.09 ab	37.79 b	39.04 bc
Ch (100 ppm)	48.43 cd	49.93 c	351.40 b	361.91 bc	34.77 c	35.99 d
Ch (200 ppm)	47.97 d	51.67 bc	357.60 b	385.00 ab	35.48 bc	38.66 bc
TRIA (2.5 ppm)	49.67 cd	52.80 ab	355.13 b	377.60 ab	36.62 bc	39.31 bc
TRIA (5 ppm)	51.90 ab	53.53 a	373.93 a	386.23 a	41.31 a	42.84 a
KSi (100 ppm)	48.17 d	52.17 ab	355.83 b	385.54 a	36.46 bc	40.00 b
KSi (200 ppm)	50.10 bc	53.93 a	360.27 ab	387.89 a	35.94 bc	39.16 bc

Means with different letters for each plant parameter are considered significantly different ($p<0.05$) using the Duncan's multiple range test.

Table 2 - The number of branches, number of leaves, and leaves area of snap beans affected by 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), and potassium silicate (KSi) in the 2021 and 2022 seasons

Treatment	No. of branches plant^{-1}		No. of leaves plant^{-1}		Leaves area (cm^2)	
	2021	2022	2021	2022	2021	2022
Control	6.67 c	6.67 b	20.00 f	20.67 c	2712.00 e	2793.67 e
6-BA (20 ppm)	7.33 abc	7.33 ab	21.33 cde	22.33 b	2901.33 d	3017.00 cd
6-BA (40 ppm)	8.33 a	8.33 a	23.33 a	24.00 a	3144.67 a	3292.67 a
Ch (100 ppm)	7.00 bc	7.00 ab	20.33 ef	20.67 c	2845.00 d	2930.33 de
Ch (200 ppm)	7.33 abc	8.00 ab	21.00 def	22.67 ab	2992.00 c	3222.00 ab
TRIA (2.5 ppm)	7.67 abc	8.33 a	21.67 bcd	23.33 ab	3096.67 ab	3237.33 ab
TRIA (5 ppm)	8.00 ab	8.33 a	22.67 ab	23.67 ab	3130.67 a	3230.67 ab
KSi (100 ppm)	7.67 abc	8.33 a	21.33 cde	23.33 ab	2886.67 d	3127.00 bc
KSi (200 ppm)	7.67 abc	8.33 a	22.33 abc	24.00 a	3018.00 bc	3250.00 ab

Means with different letters for each plant parameter are considered significantly different ($p<0.05$) using the Duncan's multiple range test.

2020; Abouelsaad and Brengi, 2022). Additionally, Ch, a biopolymer, employed in crops production primarily owing to its biocompatible and biodegradable nature, together with its notable biological activity (Hidangmayum *et al*., 2019). Despite not being a constituent of plant tissues, Ch significantly boosts the development and growth of plants (Stasińska-Jakubas and Hawrylak-Nowak, 2022). This was confirmed by El-Miniawy *et al*. (2013), who claimed that spraying Ch increased the growth (height, leaf area, and weight) of strawberry cv. Sweet Charlie. In another study, foliar application of Ch increased both the growth and nitrate reductase activity of okra (Mondal *et al*., 2012). Recently, ample evidence has shown that Si is a key nutrient for crops such as grains, legumes, and vegetables (Souri *et al*., 2021). Both *in vitro* and field investigations confirmed the favorable benefits of Si in boosting plant development, especially in stressful situations (Zargar *et al*., 2019; Souri *et al*., 2021). According to Eneji *et al*. (2008), Si has been shown to act as a bioregulator and have the capacity to enhance plant development. The usage of KSihas shown a substantial influence on the growth of several agricultural crops such as maize and strawberry (Yaghubi *et al*., 2019; Ibrahim*et al*., 2020; Gomaa *et al*., 2021).

Nevertheless, within the range of applied treatments, it was observed that the application of 5 ppm TRIA had a more pronounced impact on plant growth over both seasons. As average for the two growing seasons, with 5 ppm TRIA the snap bean height boosted by 17.23%, the leaf area expanded by

15.54%, the snap bean fresh weight boosted by 27.76%, and the shoot dry weight boosted by 38.58% as compared to the control. Triacontanol (TRIA) is plant growth regulator that has a significant function in facilitating many plants metabolic processes, ultimately resulting in enhanced growth and development (Naeem *et al.*, 2012; Islam and Mohammad, 2020). Its foliar application at low concentrations stimulates the plant biomass of the crops under both control and stressful circumstances (Naeem *et al*., 2012). A growing body of research has shown that TRIA is an important factor in controlling a wide range of plant morphological responses. One notable effect is its ability to promote many aspects of plant growth, such as increased height, enhanced biomass, greater leaf number, and expanded leaf area across multiple crop species (Naeem *et al*., 2012). This increase in plant growth might be because TRIA activates L (+)-adenosine, a second messenger that sends signals throughout the plant to boost growth by promoting cell expansion and proliferation (Masroor *et al*., 2006; Naeem *et al*., 2012).

The growth of plants is greatly impacted by the level of photosynthetic pigments, that is critical for photosynthesis. In the current study, the use of spraying treatments has resulted in enhancements in chlorophyll contents, but these improvements were seen at comparable levels in most instances (Table 3). Studies have also shown the effect of CKs, Ch, TRIA, orKSi on increasing the content of photosynthetic pigments. Cytokinins can impede or decelerate the process of plant senescence by inhibiting the degradation of chlorophyll, hence preserving the green color of the leaves (Werner and Schmülling, 2009; Kieber and Schaller, 2014). Meanwhile, treating wheat leaves with 6-BA has been shown to boost the production of the chlorophyll founder, D-aminolevulinic acid (Wang *et al.*, 2022). Also, the spray of Ch has been reported to boost the levels of photosynthetic pigments in rice plants (Pongprayoon *et al*., 2013) and creeping bentgrass plants suffering temperature stress conditions (Huang *et al*., 2021). In another study, TRIA shown a notable increase in pigment content, namely chlorophyll a, b, and carotenoids, by 25.6, 33.9, and 13.0% respectively, in the leaves of basil plants, relative to the control (Hashmi *et al*., 2011). Also, Masroor *et al*. (2006) showed similar results in their study, where they noted a substantial rise in chlorophyll and carotenoid content in tomato seedlings that were treated with TRIA. Former research has also verified the beneficial influence of KSi on the chlorophyll levels in plant leaves (Yaghubi *et al*., 2019; Zargar *et al*., 2019; Tejada-Ruiz *et al*., 2020; Gomaa *et al*., 2021).

Table 3 - Relative chlorophyll content (SPAD value) of snap bean as affected by 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), and potassium silicate (KSi) in the 2021 and 2022 seasons

Treatment	Relative chlorophyll content (SPAD value)	
	2021	2022
Control	41.33 c	41.67 c
6-BA (20 ppm)	41.67 bc	43.00 b
6-BA (40 ppm)	42.67 a	43.67 ab
Ch (100 ppm)	41.67 bc	43.33 ab
Ch (200 ppm)	42.33 ab	43.67 ab
TRIA (2.5 ppm)	42.67 a	43.33 ab
TRIA (5 ppm)	43.00 a	44.00 a
KSi (100 ppm)	42.67 a	43.00 b
KSi (200 ppm)	42.67 a	43.67 ab

Means with different letters for each plant parameter are considered significantly different ($p<0.05$) using the Duncan's multiple range test.

Elemental analysis

Nutrients are fundamental for the growth and productivity of agricultural crops. They are needed in varying quantities and play key functions in various biological processes. The application of 6-BA, Ch, TRIA, or KSi contributed to a higher level of macronutrients and micronutrients in snap bean leaves, with some exceptions (Tables 4 and 5). For instance, the treatments with 100 ppm Ch during the first season, 200 ppm Ch during the second season, and 100 ppm Si throughout both seasons demonstrated a P level comparable to that of the control. Additionally, the application of Ch at concentrations of 100 and 200 ppm resulted in limited changes to the Ca and Zn content of the leaves. Previous studies have also documented the positive effects of CKs and Si on the content and uptake of essential nutrients. Cytokinins regulate the plants' capacity to uptake various elements, like N, P, and K (Argueso *et al*., 2009). In a study conducted by Abouelsaad and Brengi (2022), the application of CKs through foliar means led to a rise in the N and P levels in potato leaves, relative to the control. Haberer and Kieber (2002) reported that CKs regulate the expression of multiple transporter genes, thereby influencing the plant's ability to uptake nutrients. From this perspective, some studies also showed that Si treatment boosts macronutri-

Table 4 - Nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca) of snap bean leavesas affected by 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), and potassium silicate (KSi) in the 2021 and 2022 seasons

Treatment	N (%)		P (%)		K (%)		Ca (%)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	3.20 g	3.17 g	0.48 f	0.50 e	2.67 e	2.69 f	2.08 f	2.12 d
6-BA (20 ppm)	3.30 f	3.25 f	0.54 b	0.53 bcd	2.86 d	2.81 e	2.15 def	2.17 c
6-BA (40 ppm)	3.40 bc	3.36 c	0.58 a	0.56 a	3.06 b	2.99 c	2.27 bc	2.22 b
Ch (100 ppm)	3.31 ef	3.29 e	0.49 ef	0.48 f	2.89 d	2.92 d	2.08 f	2.12 d
Ch (200 ppm)	3.37 cd	3.36 c	0.51 cde	0.51 e	2.92 cd	2.94 cd	2.12 ef	2.10 d
TRIA (2.5 ppm)	3.43 b	3.42 b	0.51 cde	0.53 cd	3.10 b	3.16 ab	2.31 b	2.33 a
TRIA (5 ppm)	3.48 a	3.47 a	0.53 bc	0.55 ab	3.25 a	3.21 a	2.40 a	2.36 a
KSi (100 ppm)	3.31 f	3.33 d	0.50 def	0.52 de	2.95 cd	2.93 cd	2.19 de	2.22 b
KSi (200 ppm)	3.36 de	3.38 c	0.52 bcd	0.54 abc	3.02 bc	3.11 b	2.21 cd	2.24 b

Means with different letters for each plant parameter are considered significantly different ($p<0.05$) using the Duncan's multiple range test.

Table 5 - Manganese (Mn), iron (Fe), zinc (Zn), and manganese (Mn) of snap bean leavesas affected by 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), and potassium silicate (KSi) in the 2021 and 2022 seasons

Treatment	Mg (%)		Fe (%)		Zn (%)		Mn (%)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	0.36 e	0.39 g	127.67 d	122.33f	37.33 e	40.33 e	48.33 d	50.00 e
6-BA (20 ppm)	0.41 d	0.43 f	151.33 ab	146.33 c	48.00 b	50.67 b	51.00 cd	52.67 d
6-BA (40 ppm)	0.49 ab	0.47 cd	159.00a	154.67 a	54.00 a	52.00 ab	55.33 ab	56.00 a
Ch (100 ppm)	0.44 cd	0.45 e	137.00 cd	135.00 e	41.00 de	41.67 de	51.33 c	50.00 e
Ch (200 ppm)	0.48 abc	0.49 bc	141.00 c	139.00 de	43.00 cd	42.00 de	55.67 a	54.00 abcd
TRIA (2.5 ppm)	0.48 ab	0.49 bc	155.67 a	152.33 ab	48.67 b	50.33 b	52.33 c	53.00 cd
TRIA (5 ppm)	0.51 a	0.53 a	160.67 a	156.67 a	53.67 a	54.33 a	57.00 a	55.33 ab
KSi (100 ppm)	0.45 bc	0.47 de	139.33 c	142.00 cd	43.00 cd	44.00 cd	52.67 bc	53.67 bcd
KSi (200 ppm)	0.49 a	0.50 b	145.67 bc	147.00 bc	47.00 bc	45.33 c	56.67 a	55.00 abc

Means with different letters for each plant parameter are considered significantly different ($p<0.05$) using the Duncan's multiple range test.

ent (e.g., P, K, and Ca) and micronutrient (e.g., Cu, and Fe) absorption in crops (Zargar *et al*., 2019).

The results also showed that 5 ppm TRIA significantly raised the average contents of N (9.10%), P (10.2%), K (32.73%), Ca (13.33%), Mg (38.66%), Zn (39.06%), Fe (26.8%), and Mn (14.23%), compared to the control, throughout the two successive growing seasons (Tables 4 and 5). Notably, this treatment was the most effective among the spraying treatments for all the examined nutrients. As previously reported, the use of TRIA demonstrated a significant influence on the levels of N, P, and K in some crops (Masroor *et al*., 2006; Naeem *et al*., 2012; Islam and Mohammad, 2020). Despite limited research on the impact of TRIA on micronutrient content, it may be inferred that TRIA induces modifications in plants, resulting in changed nutrient contents. In a manner similar to the 5 ppm TRIA treatment, the application of 40 ppm of 6-BA revealed the highest content of nutrients, but only for P, Fe, Zn, and Mn (Tables 4 and 5).

Flowering characteristics

Flowering characteristics (e.g., number of flowers and inflorescences) can have a great influence on the productivity of crops. In this study, the applied treatments had a beneficial effect on the length of the inflorescence in comparison to the control treatment, and the 40 ppm 6-BA treatment achieved

the highest value in both seasons (Table 6). Additionally, except for 100 ppm Ch (first season) and 20 ppm 6-BA (second season), the foliar treatments had a stimulating impact on the number of inflorescences plant^{-1} (Table 6). While there is less documentation on the impact of Ch and KSi on promoting vegetable flowering, it has been shown to have positive effects on flower crops (Pichyangkura and Chadchawan, 2015; Amer, 2020). Among the applied treatments in this study, the use of 40 ppm 6-BA, 5 ppm TRIA, and 200 ppm KSi resulted in the most significant increase in the number of flowers in the inflorescence and number of flowers plant^{-1}, a trend that persisted over both seasons. Several studies have shown the role of CKs as pivotal regulators of inflorescence morphology in plants, primarily through regulating meristem activity (Kieber and Schaller, 2014). According to D'Aloia *et al*. (2011), flowering is induced in Arabidopsis plants by exogenous CKs applied during non-inductive short days. Similar findings were reported by Rylott and Smith (1990), who demonstrated that synthetic CKs enhance plant productivity and promote competition between generative and vegetative organs.

In the current study, the number of flowers in the inflorescence and the number of flowers plant^{-1} exhibited respective increases of 22.8% and 50.15% in snap bean treated with 5 ppm TRIA, relative to the control treatment (Fig. 2). The application of TRIA has been found to exert a positive influence on the flowering process of various crops. This was confirmed by Baba *et al*. (2017), who clarified that TRIA raised the number of flowers plant^{-1} while also influencing the timing of flowering in strawberry cv. Camarosa. In addition, Sharma *et al.* (2011) tested the effects of TRIA on olives and found that it enhanced the number of flowers in relation to the control. However, some treatments, such as the application of 100 ppm Ch and 20 ppm 6-BA, did not have any notable influence on the number of flowers for the inflorescence

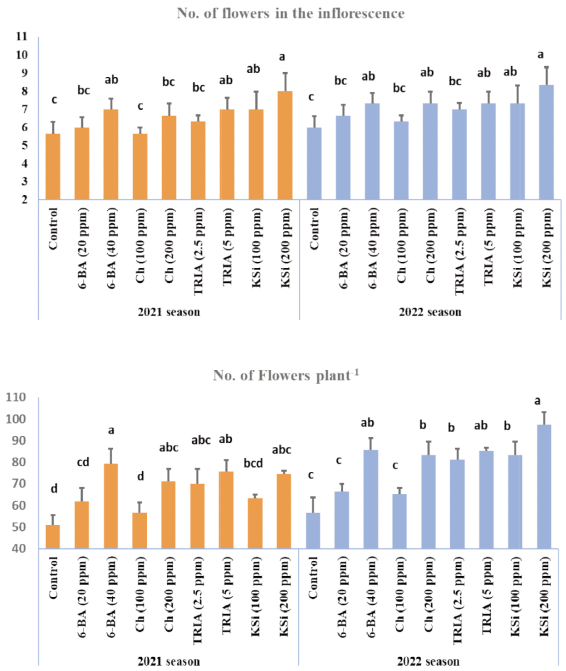

Fig. 2 - The number of flowers in the inflorescence and number of flowers plant^{-1} of snap bean as affected by 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), and potassium silicate (KSi) in the two studied seasons (summer 2021 and 2022). Means (bars) with different letters for each season are considered significantly different ($p<0.05$) using the Duncan's multiple range test. Data are mean value ± SE.

Table 6 - Number of inflorescences and length of inflorescenceof snap bean as affected by 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), and potassium silicate (KSi) in the 2021 and 2022 seasons

Treatment	No. of inflorescences plant^{-1}		Length of inflorescence (cm)	
	2021	2022	2021	2022
Control	9.00 c	9.33 c	7.50 f	7.96 f
6-BA (20 ppm)	10.33 ab	10.00 bc	10.63 cd	10.87 c
6-BA (40 ppm)	11.33 a	11.67 a	13.53 a	12.93 a
Ch (100 ppm)	10.00 bc	10.33 b	8.83 e	9.53 de
Ch (200 ppm)	10.67 ab	11.33 a	9.83 d	9.93 d
TRIA (2.5 ppm)	11.00 ab	11.67 a	10.83 c	11.20 c
TRIA (5 ppm)	11.33 a	11.67 a	12.43 b	11.77 b
KSi (100 ppm)	10.33 ab	11.33 a	8.87 e	9.30 e
KSi (200 ppm)	11.33 a	11.67 a	9.83 d	9.66 de

Means with different letters for each plant parameter are considered significantly different ($p<0.05$) using the Duncan's multiple range test.

and number of flowers plant^{-1}in relation to the control plants in both cultivation seasons (Table 6).

Snap bean yield

The value of crop yield is determined by the marketable yield, which is a crucial indicator of agricultural productivity. In this study, relative to the control, it was noted that all the evaluated treatments had a positive impact on the total pod number plant^{-1}, except the treatments with Ch at 100 ppm or KSi at 100 ppm for only the first season (Table 7). Also, the implemented treatments caused a significant increase in the number of marketable pods plant^{-1}, the weight of total fresh pods plant^{-1}, and the weight of marketable pods plant^{-1}.

Among the treatments used, applying 6-BA at a concentration of 40 ppm was the most effective treatment to achieve the highest yield parameters, a tendency that held across both seasons. This caused an increase in the total pod number by 36.67%, the number of marketable pods by 49.97%, the weight of total fresh pods by 38.23%, and the weight of marketable pods by 49.49% compared to the control treatment (Table 7). According to Jameson and Song (2016), an elevated concentration of CK throughout the developmental stages of pods and seeds has been identified as a constraining factor in their growth and maturation. A study by Nonokawa *et al.* (2012) also illustrated that CKfor both lupin and soybean crops stopped flower abortion and improved pod set, which ultimately led to a higher yield. Nonetheless, the current data showed that the treatments with 200 ppm Ch, 5 ppm TRIA, and 200 ppm KSi had comparable outcomes to the 40 ppm 6-BA treatment in terms of the number and weight of pods suitable for sale in both seasons (Table 7).

Considering the data shown in Table 8, except for the application of 6-BA at 20 ppm and KSi at 200 ppm during the first season, all treatments exhibited enhancement in the weight of the pods. The application of 6-BA (20 or 40 ppm) and TRIA at 5 ppm, resulted in statistically significant increases in pod length in both growing seasons. Furthermore, the application of Ch at 200 ppm resulted in a notable enhancement of the pod diameter during both growing seasons. Moreover, the implementation of 6-BA (20 or 40 ppm), TRIA (2.5 or 5 ppm), and KSi (100 or 200 ppm) exhibited a significant increase in pod diameter, specifically during the first growing season (Table 8). Similar studies have shown strong evidence supporting the efficacy of Ch, TRIA, and KSi applications for improving the yield and yield components of both vegetable and grain crops (Artyszak, 2018; Kocięcka and Liberacki, 2021).

Green pod quality

The value of yield quality extends beyond mere productivity. It encompasses economic, environmental, social, and health aspects, making it a crucial factor for agricultural production (Abouelsaad *et al*., 2022). As shown in Table 9, the effects of treatments on the quality features (ascorbic acid, fiber, soluble

Table 7 - Number of total pods, number of marketable pods, fresh pods weight, and marketable pods weight of snap bean as affected by 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), and potassium silicate (KSi) in the 2021 and 2022 seasons

Treatment	No. of total pods plant^{-1}		No. of marketable pods plant^{-1}		Fresh pods weight (g plant^{-1})		Marketable pods weight (g plant^{-1})	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	19.00 f	17.67 e	9.43 e	9.33 d	100.08 g	92.69 e	49.71 d	48.95 d
6-BA (20 ppm)	22.33 bcd	21.00 d	12.67 bcd	11.67 c	118.65 bcd	110.61d	66.38 bc	61.45 c
6-BA (40 ppm)	25.67 a	24.33 a	14.00 a	14.00 a	137.06 a	129.13 a	73.20 a	74.29 a
Ch (100 ppm)	20.00 ef	22 bcd	2.00 d	12.00 c	106.73 fg	116.9 bcd	64.04 c	63.76 c
Ch (200 ppm)	21.00 cde	22 bcd	13.33 abc	13.67 ab	112.14 def	116.68 bcd	71.21 ab	72.48 ab
TRIA (2.5 ppm)	22.67 bc	22.67 bc	12.33 cd	12.67 bc	121.11 bc	120.36 bc	65.90 bc	67.26 bc
TRIA (5 ppm)	23.67 b	23.33 ab	13.67 ab	13.33 ab	126.77 b	123.98 ab	72.22 ab	70.84 ab
KSi (100 ppm)	20.67 def	21.67 cd	12.00 d	12.67 bc	109.87 ef	115.19 cd	63.79 c	67.34 bc
KSi (200 ppm)	22.00 bcd	23 abc	13.67 ab	13.33 ab	117.99 cde	122.36 abc	75.09 a	70.93 ab

Means with different letters for each plant parameter are considered significantly different ($p<0.05$) using the Duncan's multiple range test.

Table 8 - Average of pod weight, pod length, fresh pods weight, and pods diameter of snap bean as affected by 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), and potassium silicate (KSi) in the 2021 and 2022 seasons

Treatment	Average of pod weight (g)		Pod length (cm)		Pods diameter (mm)	
	2021	2022	2021	2022	2021	2022
Control	5.27 b	5.25 c	12.97 b	13.27 cd	6.67d	7.67 b
6-BA (20 ppm)	5.31 ab	5.27 b	13.57 a	13.70 ab	8.00 abc	8.00 ab
6-BA (40 ppm)	5.34 a	5.31 a	13.70 a	13.77 a	8.33 ab	8.33 ab
Ch (100 ppm)	5.34 a	5.31 a	12.97 b	13.23 d	7.33 cd	8.33 ab
Ch (200 ppm)	5.34 a	5.30 a	13.10 b	13.30 cd	8.33 ab	8.67 a
TRIA (2.5 ppm)	5.34 a	5.31 a	13.40 ab	13.50 bc	8.00 abc	8.33 ab
TRIA (5 ppm)	5.36 a	5.31 a	13.57 a	13.67 ab	8.33 ab	8.33 ab
KSi (100 ppm)	5.32 ab	5.32 a	13.07 b	13.33 cd	7.67 ab	8.00 ab
KSi (200 ppm)	5.36 a	5.32 a	13.37 ab	13.47 bcd	8.67a	8.33 ab

Means with different letters for each plant parameter are considered significantly different ($p<0.05$) using the Duncan's multiple range test.

Table 9 - The contents of ascorbic acid, fiber, soluble sugar, and protein in snap bean pods as affected by 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), and potassium silicate (KSi) in the 2021 and 2022 seasons

Treatment	Ascorbic acid (mg 100 g FW^{-1})		Fiber (g 100 g FW^{-1})		Soluble sugar (g 100 g FW^{-1})		Protein (%)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	17.43 d	18.60 c	3.51a	3.49 a	2.13 d	2.19 c	17.94 f	18.25 e
6-BA (20 ppm)	18.53 cd	19.73 bc	3.36 c	3.35 d	2.21 c	2.19 c	18.69 de	19.31 c
6-BA (40 ppm)	19.57 abc	20.38 ab	3.31 d	3.31 e	2.25 b	2.23 b	19.75 ab	19.44 c
Ch (100 ppm)	19.70 abc	20.13 b	3.38 bc	3.36 d	2.20 c	2.21 bc	18.06 f	18.81 d
Ch (200 ppm)	20.70 a	21.30 a	3.39 bc	3.37 cd	2.21 c	2.21 bc	19.13 cd	19.44 c
TRIA (2.5 ppm)	19.31 bc	19.78 b	3.30 d	3.29 e	2.30 a	2.32 a	19.63 abc	19.88 b
TRIA (5 ppm)	20.44 ab	20.61 ab	3.28 d	3.28 e	2.33 a	2.32 a	20.00 a	20.31 a
KSi (100 ppm)	19.70 abc	20.07 b	3.40 bc	3.39 bc	2.20 c	2.21 bc	18.50 ef	18.81 d
KSi (200 ppm)	19.87 abc	20.07 b	3.42 b	3.41 b	2.21 bc	2.23 b	19.13 cd	19.63 bc

Means with different letters for each plant parameter are considered significantly different ($p<0.05$) using the Duncan's multiple range test.

sugar, and protein) of snap bean green pods were investigated. Except for plants treated with 6-BA at 20 ppm, the ascorbic acid content in the pods of treated plants was significantly increased with respect to the control in both cultivation seasons. Also, the treatments with 6-BA (40 ppm), TRIA (2.5 or 5 ppm), or KSi (200 ppm) significantly increased the amount of soluble sugar in the pods with respect to the control in both cultivation seasons. Moreover, it was observed that the application of 6-BA (20 and 40 ppm), Ch (200 ppm), TRIA (2.5 or 5 ppm), orKSi (200 ppm) resulted in enhancement of protein content within the pods, with respect to the control plants, across both seasons (Table 9).Comparable findings also demonstrated the beneficial effects of Cks, Ch, TRIA, orKSi on the levels of protein, soluble sugar, and ascorbic acid in cereal crops, vegetable, or legumes (Naeem *et al*., 2012; Artyszak, 2018; Hu *et al*., 2022). Moreover, snap beans should have fleshier green pods with little fiber content where immature pods are eaten as vegetables. In this study, the applied treatments significantly reduced the amount of fiber in the pods relative to the control group (Table 9). Overall, in both growth seasons, the TRIA (5 ppm) spraying treatment showed remarkable efficacy across all quality criteria.

4. Conclusions

The applications of 6-benzylaminopurine (6-BA), chitosan (Ch), triacontanol (TRIA), or potassium silicate (KSi) by foliar spraying have the potential to enhance the development and agronomic characteristics of snap bean plants.Specifically, the use of 5 ppm TRIA demonstrates the most advantageous improvements in growth, blooming, yield, and overall quality. This study could potentially establish a theoretical framework for improving the commercial production of snap beans in summer conditions. Also, by demonstrating the efficacy of biostimulants, sustainable agricultural practices can enhance food production and environmental stewardship in subtropical regions.

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Pigment composition and physico-chemical parameters of Bittergourd (*Momordica charantia* L. cv. Jadeite) during postharvest period as influenced by illumination colors

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Abstract: Fruits and vegetables that exhibit a higher chlorophyll content, as reflected in their visual appearance, are the preferred choice of consumers. The study aimed to evaluate the effects of Light Emitting Diodes (LEDs) on the physical, chemical, and pigmentation quality of bitter gourd using white, blue, and red at 1.5 W/135 lumens (Foshan Electrical and Lighting Co., Ltd [FSL], China). Bitter gourd, with a short postharvest life of 4-5 days due to physical and chemical disorders, was harvested weighing 300–400 g and 25 x 5 cm from the farm and subjected to varying illuminations within a 4-hour period for five days, with measurements taken daily. Statistical differences between treatments were observed in physicochemical parameters such as fruit shrivelling, yellowing, visual appearance, weight loss, dry matter content, total chlorophyll, pH values, and TA. The quality and shelf-life of bitter gourd fruits were found to be improved by the white LED. The visual appearance was maintained, and fruit shrivelling and yellowing were delayed, with lower weight loss observed. Slight changes in chlorophylls and carotenoids, vitamin C, and a shelf-life of 5 days were recorded.

1. Introduction

Momordica charantia L. is known as bitter gourd, with its good nutritional and medicinal properties, is grown in approximately 340,000 ha (Dhillon *et al*., 2016) annually in tropical Asia, including Eastern Asia, India, and China, which is its center of origin (Behera *et al.*, 2010;

Prajapati *et al*., 2021 a). The production volume in the Philippines has increased by 1.8% to 32.05 thousand metric tons (PSA, 2022). Its primary demand arises from its anti-diabetic properties (Rosario and Macusi, 2009). The presence of phenols, terpenes, and flavonoids in bitter gourd contributes to its bitter taste and antioxidant properties (Dutta *et al*., 2021), which are desired by consumers (Behera *et al.*, 2010; Taiti *et al*., 2017) and can help prolong its shelf-life (Salas *et al*., 2015). The metabolites found in bitter gourd are affected by cultivars and cultural practices such as Light, temperature, soil, and nutrition (Valyaie *et al*., 2021). The importance of Light in postharvest produce cannot be overstated, as plants perceive stimulus for growth and development through light. Recent studies have discovered that even after plants are harvested, their light-dependent processes continue. To ensure the longevity of postharvest fruits and vegetables and maintain their metabolite levels, various treatments - both chemical and non-chemical - can be applied. For example, bittergourd fruits can be stored for up to five days under ambient conditions without ripening, yellowing, or losing their bitterness (Salas *et al.*, 2015; Prajapati *et al*., 2021 a). As a means of preserving postharvest produce, researchers have begun exploring the use of Light Emitting Diodes (LEDs). In fact, supplemental lighting from LEDs has been shown to enhance the market value of harvested sweet peppers by inducing colour break (Jones, 2018).

Researchers have therefore explored the use of Light Emitting Diodes (LEDs) to prolong shelf-life and maintain metabolites in various crops (Ma *et al*., 2014; D'Souza *et al*., 2015; Bantis *et al*., 2018; Loi *et al.*, 2020; Poonia *et al*., 2022). The findings of these studies have been corroborated by the upregulation of vitamin C, antioxidants, anthocyanins, and the physical appearance of fresh produce towards market acceptability. For example, white LED lighting facilitates the accumulation of phenols in harvested vegetables (Poonia *et al*., 2022), which are known to have antioxidant and anti-inflammatory properties and may enhance the health benefits of these crops. It was observed that the LEDs had a drying effect, leading to a rapid increase in transpiration, as reported by Chua *et al*. (2021). Various studies have shown that different types of light can have varying effects on different types of vegetables. For example, white and blue LEDs can modulate the stomata opening and number of stomates (Zhan *et al*., 2013), resulting in weight loss of certain vegetables such as *Brassica oleracea* L. var. *italica* Plenck (Favre *et al*., 2018), *Brassica oleracea* var. chinensis Lei (Zhou *et al*., 2020), and freshly-cut leaves of *Amaranthus dubius* L. (Jin *et al*., 2021). On the other hand, dark-stored celery has a lower dry matter content compared to fresh-cut celery due to lower total soluble solids, ascorbic acid, and chlorophylls (Zhan *et al*., 2013; Florkowski *et al*., 2014). In another study, freshly sliced cherry tomatoes exposed to white, blue, and green LEDs showed a transitory increase in vitamin C one day after slicing, while those exposed to red light remained stable (Kong *et al*., 2020).

It is worth noting that the effect of LED lighting on postharvest crops varies depending on the type of crop, LED intensity, and duration of exposure. For example, in previous studies, broccoli exposed to 9.5 and 19.0 W m^{-2} white LED illumination for three hours per day showed delayed chlorophyll degradation and lower weight loss (Pintos *et al*., 2020). Okra fruits treated with white and blue LEDs (17.28 W m^{-2}) for 8 h showed an increased total phenolics content (Thilini Deepashika Perera *et al*., 2022), while berry grape treated with 41 and 42 W m^{-2} of LED showed an increased anthocyanin content at 24 h, and blue LED decreased fungal infections in citrus fruits at a fluence rate of 120 W m^{-2} and 700 W m^{-2} for 18 h under 25°C (Nassarawa *et al*., 2020). In addition, bitter gourd exposed to UV-C LED for 40 min at 10°C and 85-95% RH had a prolonged shelf-life of up to 16 d (Prajapati *et al*., 2021 b). While chemical-based treatments have been the focus of many researchers to improve the postharvest life of vegetables, there is a growing interest in using LED treatments to increase shelf-life and enhance metabolites. It is hypothesized that varying illuminations of white, blue, and red LED with 1.5 Wattage (W) can delay pigmental degradation, preserve organic compounds, and extend shelf-life in harvested bitter gourd. This study tried to assess how the quality of bitter gourd change during post-harvest by applying varying illuminations color LED (white, blue, and red) with 1.5 Wattage (W).

2. Materials and Methods

Fruit samples and sample preparation

The cultural practices and harvesting process of sixty bitter gourds from a commercial vegetable farm

in Barangay Buenavista, Baybay City, Leyte (lat. 10° 39' 34.0" N, long. 124° 50' 27.6" E) are described in this study. Organic and inorganic fertilizers were applied, including chicken dung through the basal method and soil drenching for urea and complete fertilizers during the vegetative and reproductive stages. The fruits, weighing 300-400 g and measuring 25 x 5 cm, were harvested at the dark green stage and were carried out carefully to minimize mechanical injuries. Fruits with defects were excluded from the study. The farmer-harvester, an expert in harvesting, chose the fruits for marketing based on the local market standard from the City mentioned above. The fruits were then transported from the farm to the Department of Horticulture Crop Physiology Laboratory for postharvest assessment from October 28 to November 03, 2022. The shelf-life of the fruits was 5 days (d), and the treatment application was done after 24 hours (h) to acclimatize and equilibrate the fruits at ambient conditions. The amount and time of fertilizer application were not included in the present study, as it was based on the farmer's feedback during the harvest since the study utilized fruits from the farm instead of from the wet market.

Treatment preparation

The cardboard boxes with aluminum foil were arranged on the surface and equipped with 1.5 W/135 lumens (Foshan Electrical and Lighting Co., Ltd [FSL], China) LED lights, measuring 56 [L] x 45 [W] x 24 [H] cm. The LED source was positioned at a distance of 19 cm from the fruits. In the current study, each treatment was subjected to white, blue, and red LEDs for a duration of 4 h, with five (5) fruit samples per treatment. After incubation, the lights inside each cardboard box were switched off. However, the irradiated fruit samples were transferred and placed in plastic trays (40 × 30 cm) under ambient conditions (26-28°C). Each tray represented a replicate exposed to lights that remained switched on continuously for 8 h daily, including non-incubated fruit samples for storage and postharvest evaluation. Enhanced and stable metabolites were demonstrated during storage, resulting in a prolonged shelf-life from exposure to UV-C LED for 40 minutes (min), blue and red LEDs for 24 h, white and blue light for 8 h, and white LED for 3 h daily, as shown in previous studies (Nassarawa *et al*., 2020; Pintos *et al*., 2020; Prajapati *et al*., 2021 b; Thilini Deepashika Perera *et al*., 2022).

Experimental design

In a completely randomized design (CRD), the study had five samples per treatment replicated three times. The treatments were designated as follows:

T1 - Control (Room light condition),
T2 - Incubated 4 h with white LED at 1.5 Watt/135 lumen (FSL, China),
T3 - Incubated 4 h with blue LED at 1.5 Watt/135 lumen (FSL, China),
T4 - Incubated 4 h with red LED at 1.5 Watt/135 lumen (FSL, China).

Data collection

Physico-chemical parameters. The fruits were assessed before and after gathering using a digital weighing scale (General Master, Japan), with the parameters being determined for each fruit from the five samples in replication manually every day for six days using different indices.

The cumulative weight loss from the five samples from each replication was determined by weighing the initial weight and daily as known storage period (Prajapati *et al*., 2021 b).

The fruit shrivelling index was assessed with slight modifications (Benitez *et al*., 2015; Lualhati and Del Carmen, 2018) using a 4-point scale ranging from 1 to 4 (where 1 indicated no shrivelling, 2 indicated slight shrivelling (1-25% fruit surface affected), 3 indicated moderate shrivelling (26-50% fruit surface affected), and 4 indicated severe shrivelling (more than 50% fruit surface affected).

The visual quality rating (VQR) of Bitter gourd was evaluated daily as reported by Valida *et al*. (2018). In brief, VQR was assessed using a 9-point scale, where 9 indicated excellent, field fresh or no defects, 7 indicated good, defects minor, 5 indicated fair, defects moderate, limit of marketability, 3 indicated poor, defects serious, limit of edibility, and 1 indicated non-edible under usual condition.

The degree of yellowing was rated manually daily as reported by Valida *et al.* (2018) using a 5-point scale, where 1 indicated full green, 2 indicated 1-10% surface yellowing, 3 indicated 11-30% surface yellowing, 4 indicated 31-50% surface yellowing, and 5 indicated extensive yellowing/discoloration.

The dry matter content (%) was determined by subjecting the samples (50 g) to oven drying at 70°C for 24 h until they reached a constant weight. The remaining weight of the samples after drying served

as input to calculate the percent dry matter content as a percentage of the wet sample (Gonzales and Benitez, 2019).

The pigment composition (mg g^{-1}) was determined by soaking a gram of each representative bitter gourd fruit in 10 ml of 95% ethanol overnight. The absorbance of the filtrates at wavelengths of 666, 653, and 470 nm was measured using an ultraviolet-visible spectrophotometer at the VSU-CASL (Salas *et al*., 2020).

The shelf life was determined when sample fruits reached a VQR of 5, which is fair with moderate defects and limited marketability (Salas *et al*., 2015; Valida *et al*., 2018).

Chemical Parameters. For the examination of chemical parameters, one fruit from each replication was randomly selected as a representative. The protocol appears to have been based on the report of Gonzales and Benitez (2019) and Salas *et al*. (2020).

The fresh-cut samples were homogenized in 10 g per 50 ml distilled water for 10 min using a homemade blender (CAMEL®), and the filtrates were measured for potential hydrogen (pH), electrical conductivity (EC) and total dissolved solids (TDS) using a smart combined meter (Milwaukee, MW 802)

Total soluble solid (°Brix) was measured using a hand-held refractometer (Atago N1, Japan) by placing 1-3 drops of juice on the instrument prism and taking the reading.

Titrable acidity (%) was determined by adding 5 ml extract with two drops of 1% phenolphthalein indicator into a volumetric flask containing 4 g NaOH diluted with 1 L distilled water, followed by titration with 0.1% NaOH until a faint pink colour was obtained.

Finally, for Vitamin C analysis (mg 100 g fresh $fruit^{-1}$), fresh cut (12 g) was subjected to 5 min with 120 ml distilled water in a blender until supernatant filtered. An aliquot (1 ml) extract was mixed in a 125 ml Erlenmeyer flask containing 50 ml distilled water and three drops of starch solution as an indicator. Iodometric titration and volumetric techniques were employed for the analysis.

Statistical analysis

After the analysis of variance (ANOVA) was conducted, the treatment mean was compared and separated by the Honest Significant Difference (HSD) using the Statistical Tool for Agricultural Research (STAR) program, which had been developed by the International Rice Research Institute (IRRI).

3. Results and Discussion

Fruit shriveling

Table 1 shows a gradually increasing index of bitter gourd fruit shriveling. At 3 days (d), delayed shriveling for those fruits with white and blue light treatments. Consequently, shriveling was slight to moderate until 6 d of storage. Endalew (2020) states that shriveled fruit reduces consumer acceptability and marketability. During the transport, the cushioning materials such as newspaper, foam nets, leaves, and other local materials then packed in cardboard boxes or plastic trays could help prevent fruits from bruising and touching, which leads to morpho-physiological disorder and increased entropy (Ahmad and Siddiqui, 2015; Valida *et al*., 2018; Hussein *et al*., 2020).

Table 1 - Fruit shrivelling of bittergourd as influenced by illumination colors during storage

Treatments	Fruit shrivelling				
	2 d	3 d	4 d	5 d	6 d
Control (No LEDs)	1.00±0.00 NS	1.06±0.12 ab	1.78±0.57 NS	2.42±0.24 NS	2.72±0.05 NS
White LEDs	1.00±0.00 NS	1.00±0.00 b	1.27±0.12 NS	2.40±0.40 NS	2.60±0.35 NS
Blue LEDs	1.00±0.00 NS	1.00±0.00 b	1.33±0.42 NS	2.40±0.40 NS	2.78±0.71 NS
Red LEDs	1.00±0.00 NS	1.21±0.03 a	1.50±0.30 NS	2.33±0.42 NS	3.13±0.23 NS
CV (%)	0.00	5.56	26.28	15.51	14.74

Data represent mean ± deviation standard.
Mean values (n = 5) from three replicates in each column of sampling times followed by different letters are significantly different from each other at 5% Tukey's test. NS= not significant.
CV= Coefficient of variation.

Yellowing index

Bitter gourd is a climacteric fruit that ripens and turns yellow during storage due to ethylene production (Yahia *et al.*, 2019). As shown in Table 2, there is a 1-10% (YI=2) yellowing after the third. Fruits exposed to white LEDs (WL) and Blue LEDs (BL) maintained the greenness at 3 d of storage but compared to control. As the days passed until 6 d, the yellowing progressed to 11-30% (YI=3), and the quality began deteriorating. Several factors, including senescence, free radicals, energy, metal ions, and some secondary metabolites in fruits and vegetables, can cause postharvest yellowing (Luo *et al*., 2019). Diaz *et al.* (2006) observed that chlorophyll degradation and anthocyanin accumulation cause the yellowing of *Arabidopsis thaliana* leaves.

According to other studies, light treatment prevents some fruit postharvest problems, for example: (a) the use of Red LED light delays yellowing and reduces ethylene production in broccoli inflorescences (Ma *et al.*, 2014); (b) white and Blue LEDs of 20 $molm^{-2}$ s^{-1} controls yellowing and maintains the green color of the outer and inner leaves of Brussels sprouts during storage (Hasperué *et al.*, 2016); (c) artificial lighting modulates stomata opening in green tissues keeps fruits from dehydration and delays degreening by delaying cell aging and disorganization (Pintos *et al*., 2020); (d) continuous exposure to white and blue LEDs under 5 and 22°C results to have higher chlorophylls a and b which remains stable and green during storage (Hasperué *et al*., 2016 a, b). It is in line with the findings of Loi *et al.* (2019), which the broccoli heads increased the chlorophylls resulted from metabolic activity exposed with BL, 467 nm, 4.1 W/m and WL, 31 lm/W.

Visual quality rating

In the selection of fruits and vegetables, visual characteristics are frequently used by consumers. Visual quality loss with moderate defects after 5 d of storage was delayed by white and blue light, as shown in Table 3. Poor quality with serious defects was observed in those fruits without LED and red LED

Table 2 - Yellowing index of bittergourd as influenced by illumination colors during storage

Treatments	Yellowing Index				
	2 d	3 d	4 d	5 d	6 d
Control (No LEDs)	1.00±0.00 NS	1.06±0.12 ab	1.91±0.38 NS	3.14±0.43 NS	3.33±0.76 NS
White LEDs	1.00±0.00 NS	1.00±0.00 b	1.40±0.40 NS	2.45±0.40 NS	3.01±0.52 NS
Blue LEDs	1.00±0.00 NS	1.13±0.12 ab	1.64±0.34 NS	2.75±0.43 NS	3.03±0.29 NS
Red LEDs	1.00±0.00 NS	1.28±0.10 a	1.98±0.23 NS	2.83±0.76 NS	3.50±0.50 NS
CV (%)	0.00	8.64	19.70	18.86	16.91

Data represent mean ± deviation standard.
Mean values (n = 5) from three replicates in each column of sampling times followed by different letters are significantly different from each other at 5% Tukey's test;NS= not significant.
CV= Coefficient of variation.

Table 3 - Visual quality rating of bittergourd as influenced by illumination colors during storage. Data represent mean ± deviation standard

Treatments	Visual quality rating				
	2 d	3 d	4 d	5 d	6 d
Control (No LEDs)	9.00±0.00 NS	7.84±0.73 NS	6.18±0.84 NS	3.85±0.13 b	3.06±0.82 NS
White LEDs	9.00±0.00 NS	8.73±0.23 NS	6.47±0.61 NS	6.24±0.80 a	4.13±0.81 NS
Blue LEDs	9.00±0.00 NS	8.73±0.46 NS	6.53±0.50 NS	5.50±0.87 ab	3.97±0.29 NS
Red LEDs	9.00±0.00 NS	8.00±0.20 NS	5.93±0.31 NS	3.71±0.92 b	3.28±1.49 NS
CV (%)	0.00	5.52	9.48	15.55	26.45

Data represent mean ± deviation standard.
Mean values (n = 5) from three replicates in each column of sampling times followed by different letters are significantly different from each other at 5% Tukey's test; NS= not significant.
CV= Coefficient of variation.

treatments, leaving them unfit for consumption (Fig. 1). The deterioration of visual quality over time following harvest has been observed in various studies. However, the visual appearance and shelf life of fresh produce can be improved by postharvest lighting using LEDs. The effect of white-blue LEDs on the outer and inner leaves of Brussels sprouts during a 10 d storage period at 22°C was investigated. Lower respiration rates and better visual quality were found

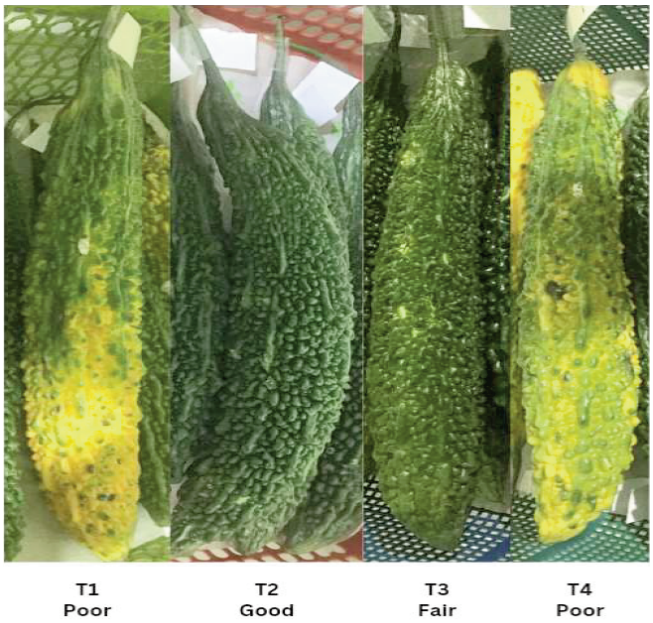

Fig. 1 - A visual quality rating (VQR) of bitter gourd fruit 'Jadeite' cultivar at 5th day of storage. (A) T1- Control (No LEDs), the fruits were poor with serious defects and limit edibility; (B) T2- White LEDs, the fruits were good with minor defects; (C) T3- Blue LEDs, the fruits were fair with moderate defects and limit marketability; (D) T4- Red LEDs, the fruits were poor with serious defects and limit edibility.

than in the controls (Poonia *et al*., 2022). The levels of vitamin C (35 mg 100 g FW^{-1}) and soluble carbohydrates (8.3 mg g FW^{-1} sucrose on the fourth day) in fresh-cut lettuce were increased by postharvest lighting. On the initial day, light at 50 % falls to 15 mg 100 g FW^{-1} vitamin C and 0.2 mg g FW^{-1} sucrose, respectively (Witkowska, 2013).

The observed improved visual appearance and, eventually, longer shelf life can be attributed to this (Bantis *et al*., 2018). As demonstrated by freshly cut leaves amaranth, photosynthesis is unsustainable with insufficient light intensity (Jin *et al*., 2021). As a signal driving this process, the conversion of sugars via gluconeogenesis is driven by low irradiance, producing glucose through the TCA cycle (Wolter and Seifu, 2015). Fresh-cut celery had a higher total dissolved solids than dark-stored celery (Zhan *et al*., 2013). It indicates that photosynthesis continued functioning, allowing postharvest crops to remain viable. Because stored organic acids such as malic and citric are undissociated from the vacuoles, preservation of titratable acidity is a good indicator of fruit quality, reflecting shelf-life (Utama *et al*., 2022). Furthermore, the activity of polyphenol oxidase, responsible for cell integrity breakdown, can be inhibited by ascorbate, making it a universal antioxidant that can help preserve the freshness of fruits and vegetables (Toivonen and Brummell, 2008).

Weight loss (%)

In Table 4, weight loss (%) is shown in increasing order. The acceptable limit (1-11%) for bitter gourd is observed in the present findings; if it exceeds, it leads to severe fruit shriveling and undesirability (Lualhati and Del Carmen, 2018). As a result, significant weight loss after three days of storage is prevented in bitter

Table 4 - Weight loss (%) and dry matter content (%) of bittergourd as influenced by illumination colors during storage

Treatments	Weight loss (%)				Dry matter content (%)
	3 d	4 d	5 d	6 d	
Control (No LEDs)	1.95±0.23 ab	4.48±0.68 NS	7.42±1.23 NS	11.41±1.42 NS	20.87±0.64 b
White LEDs	1.58±0.12 b	4.04±0.19 NS	6.52±0.08 NS	9.99±0.17 NS	20.67±0.31 b
Blue LEDs	2.15±0.20 a	4.64±0.39 NS	7.46±0.78 NS	11.26±1.11 NS	21.40±1.31 b
Red LEDs	1.88±0.12 ab	4.83±0.49 NS	7.79±0.80 NS	12.17±0.77 NS	20.47±0.46 b
Initial data	-	-	-	-	26.00±0.00 a
CV (%)	9.08	10.44	11.39	8.77	3.19

Data represent mean ± deviation standard.
Mean values (n = 5) from three replicates in each column of sampling times followed by different letters are significantly different from each other at 5% Tukey's test; NS= not significant.
CV= Coefficient of variation.

gourd treated with white and red light. The findings are consistent with Li's (2016) study on strawberries, which found that fruits illuminated with blue light lost more weight than fruits in white and dark treatments due to increased calyx transpiration under blue light. Bitter gourd wilts and shrivels due to weight loss, which lowers its market value and consumer acceptability (Prajapati, 2021 a).

Furthermore, shelf life is extended by slowing the water absorption rate, as with pepper (Wills *et al*., 1998). Weight loss in fresh produce is caused by water loss due to transpiration and respiration (Zhu *et al.,* 2008). Throughout the storage period, the percentage of weight loss increases. Typically, weight loss during fruit storage results from the respiratory process, humidity transfer, and some oxidation processes (Ayranci and Tunc, 2003). Quality parameters were preserved, and storage was prolonged from 15 to 20 d by treating bitter gourd fruits with 100 mmol L^{-1} /mM Calcium lactate (CL) at 10°C and 85-95 % RH, 1-MCP, edible coating with carnauba wax (1.0 %), UV-C LED 40 min, modified atmospheric packaging using LDPE (100 microns) (Prajapati *et al*., 2021 b). The enhanced membrane integrity was due to calcium stabilizing the cell membrane and turgor pressure. In UV-C LED exposure, the epicuticular wax morphology changed by reducing microcrystalline structure, which gave a better protective layer and thus reduced weight loss (Prajapati *et al*., 2021 a).

Dry matter content (%)

The data presented in Table 4 clearly demonstrate an inverse relationship between the moisture content and dry matter content of eggplant, both during storage and after harvest. The high respiration rate of eggplant fruit results in high water loss and low dry matter content, which can lead to shrivelling and reduced consumer acceptability, unless proper postharvest treatments are applied. In this regard, fruits treated with 1% Alginate and lacking an edible coating showed a lower dry matter content on the initial day, decreasing from 8.4% to 7.5%. Despite the positive results reported by Huang *et al.* (2017) on the dry biomass of oyster mushrooms under blue LED light, our study found that the dry matter content was unaffected by this type of light.

Ripening is a complex process that involves the accumulation of various metabolites, such as carbohydrates, sugars, vitamins, and other compounds, which contribute to the dry matter content and the conversion into other products. The fresh-cut celery treated with LEDs revealed a higher dry matter content than dark-stored celery, due to the higher levels of total soluble solids, ascorbic acid, and chlorophylls, as reported by Zhan *et al.* (2013) and Florkowski *et al.* (2014). Finally, the results are consistent with those of Bantis *et al*. (2018), who found that high levels of blue LED light exposure can increase the dry matter content and enhance radical scavengers and soluble sugars in mushrooms, thanks to the higher metabolic activity in synthesizing other products as a result of respiration.

Pigmental composition (mg g^{-1})

Bittergourd fruits before treatment contained chlorophyll a (6.57 mg g^{-1}), b (3.61 mg g^{-1}), and total (10.19 mg g^{-1}), respectively. Table 5 shows that the fruits at 5 d storage had maintaining levels of pigments but were degrading. Fruits exposed to

Table 5 - Pigmental composition (mg g^{-1}) and shelf-life (d) of bittergourd as influenced by illumination colors during storage

Treatments	Chlorophyll and carotenoid contents (mg g^{-1})				Shelf-life (d)
	Chl a	Chl b	T Chl	T Car^{NS}	
Control (No LEDs)	3.06±0.36 b	1.58±0.35 b	4.65±0.71 b	1.80±0.34 NS	4.67±0.58 NS
White LEDs	5.66±2.01 ab	2.64±1.08 ab	8.30±3.07 ab	2.53±0.58 NS	5.00±0.10 NS
Blue LEDs	4.91±1.21 ab	2.34±0.55 ab	7.25±1.75 ab	1.95±0.51 NS	5.33±0.58 NS
Red LEDs	4.56±0.85 ab	2.20±0.27 ab	6.76±1.16 ab	1.99±0.24 NS	4.67±0.58 NS
Initial data	6.57±0.00 a	3.61±0.00 a	10.19±0.00 a	2.48±0.00 NS	-
CV (%)	22.73	23.28	22.71	18.15	14.38

Data represent mean ± deviation standard.
Mean values (n = 5) from three replicates in each column of sampling times followed by different letters are significantly different from each other at 5% Tukey's test; NS= not significant.
CV= Coefficient of variation.

white, blue, and red LEDs had better pigmental composition than those exposed to control. Between the two pigments, chlorophyll a contained higher levels than chlorophyll b, resulting in reduced amounts during storage. It agrees with Prajapati *et al.* (2021 a), who found that total chlorophyll decreased with storage treatments. This event is considered a tug-of-war between two pigments during the yellowing process. High chlorophyll is sought after by consumers. The chlorophyll content decreased when the broccoli started yellowing (Loi *et al*., 2019). In contrast, there were carotenoid and chlorophyll syntheses in Brussels sprouts under low light intensity using WB LED for 10 d. However, photooxidation will destroy the carotenoid at higher light intensity (Hasperué *et al*., 2016). The delayed decline of carotenoid contents was due to exposure to white LED at 1.4 W m^{-2} for 8 d (Nassarawa *et al*., 2020). The carotenoid primary function is to act as an accessory light-harvesting system, which aids in light absorption. It is considered to be photoprotective by inhibiting free radicals in chloroplasts (Jones, 2018; Salas *et al*., 2019). It acts as an antioxidant by donating hydrogen to neutralize singlet oxygen (Gorni *et al*., 2021).

The phytochrome family perceives red light between 600 and 750 nm. At the same time, the blue light (320-500 nm) spectrum includes cryptochromes and phototropins under the ZEITLUPE/ADAGIO family. Similarly, the stability of Cryptochrome Circadian Regulator 1 (CRY1) depends on the illumination, but the Cryptochrome Circadian Regulator 2 (CRY2) receptor breaks after light exposure (Jones, 2018). Although Red LEDs (RL) did not induce chlorophyll accumulation, when combined with Blue LEDs (BL), the chlorophyll content of a non-heading Chinese cabbage increased (Fan *et al*., 2013; Bantis *et al.* 2018). LED illumination improved crop pigmental composition while lowering reactive oxygen species activity (Kong *et al*., 2020; Loi *et al*., 2020). The green pigments found in fresh vegetables and fruits originated from chlorophyll in the chloroplast, which would then destroy PSII by decreasing the chlorophyll contents during yellowing (Luo *et al*., 2019). Both chl a and chl b have collaborated to widen the light spectrum associated with photoreceptors to harvest light emissions (Salas *et al.*, 2019). Before yellowing, NYC1 levels increased, but after 5 d of storage, they decreased (Luo *et al.*, 2019). The intensity of RL at 50 $molm^{-2}$ s^{-1} increased the carotenoid content of Satsuma mandarin after 6 d of storage. At the same time, BL did not improve carotenoid accumulation (Ma *et al*., 2014; Bantis *et al*., 2018).

Shelf-life

It was found that the use of different illuminations did not have a significant impact on the shelf life of bitter gourd, as per the study that was discussed (Table 5). Regardless of whether LEDs were used or not, the fruits were only lasting for around 4-5 d during storage (Salas *et al.*, 2015). However, it was suggested that future research could explore the possibility of increasing LED duration and lowering temperatures in order to prolong the shelf life of bitter gourd. It was noted that fruits are generally highly perishable when stored at room temperature, which can result in significant postharvest losses (Pott *et al*., 2020). As such, extending the shelf life of such crops can be considered a significant breakthrough in research. Bitter gourd, in particular, degrades quite rapidly due to various factors, such as the presence of protruding ridges, excessive seed development, tissue softening, yellowing, and ripening, which can make it challenging to market (Prajapati *et al*., 2021 b). Previous studies have shown that LEDs can have a positive impact on the metabolites and postharvest life of various crops, including bitter gourd. For example, it was found that the shelf life of crops like *M. charantia* (Prajapati *et al*., 2021 a), *Brassica oleracea* L. var. Italica (Loi *et al.*, 2019), and broccoli (Hasperue *et al.*, 2016; Pintos *et al*., 2020) was prolonged significantly when exposed to certain types of LEDs.

It was found that the study did not yield significant results on the shelf-life of bitter gourd. However, as argued by D'Souza *et al*. (2015), the shelf-life and quality of horticultural products can be influenced by postharvest light through the potential increase of soluble carbohydrates, which serve as the substrate for respiration during postharvest storage, and through the enhancement or preservation of visual appeal through pigment accumulation, such as lycopene, carotenoids, and anthocyanins. Significant potential has been demonstrated by LED technology for promoting the growth and synthesis of beneficial compounds and extending the shelf-life of fruits and vegetables during postharvest storage, as reported by Loi *et al*. (2020). It was observed that fruits did not experience any prevention from shrivelling,

yellowing, and weight loss when exposed to blue and red LED. Additionally, there was no influence on visual quality with red LED on the fifth day. However, an increased content of total chlorophyll, vitamin C, pH, and total phenolics in cabbage was observed when exposed to continuous lights using white, blue, green, and red LEDs. Green tomatoes' delayed ripening and softening resulted from exposure to blue and red LEDs for 21 d, while an increase in contents of sugars, chlorophyll, and carotenoids in broccoli at 5°C and 22°C was observed when exposed to white and blue LED with a fluence rate of 20 W m^{-2} (reviewed by Nassarawa *et al*., 2020).

Chemical parameters

Fruit cell walls containing polysaccharides solubilized and hydrolyzed into simple sugars, which gives rise to the soluble solids (Ayu *et al*., 2020; Mirshekari *et al*., 2020; Suriati *et al*., 2022) and are affected by room temperature (Mutua *et al.*, 2021). However, the decrease in soluble solids resulted from respiration during fruit storage (De Paula *et al*., 2020). The enhanced shelf-life of fruits indicates slow utilization of soluble solids (Nassarawa *et al.*, 2020). One of the critical metabolites is Vitamin C, an antioxidant against free radicals and a nutrient source in fruits and vegetables (Loi *et al*., 2019; Mirshekari *et al*., 2020). As a precursor, glucose synthesis is necessary for synthesizing vitamin C via the L-galactose pathway and D-galacturonic acid (Fernandez and De Guzman, 2022). Using organic acids in respiration increases pH over storage (Gonzales and Benitez, 2019). The increase in pH values was concomitant with a reduction in Vitamin C and TA, which concurs with Salas *et al*. (2020). Cucumber's shelf life has been extended due to the preservation of TA over time (Zapata *et al*. 2008, as cited by Gonzales and Benitez 2019). Based on the study results, the electrical conductivity (EC) increased with storage time. Conforming to the Salas *et al*. (2020) study, eggplant fruits with a high amount of electrolytes deteriorate quickly. This is due to the gradual breakdown of cell membrane integrity mediated by lower lipoxygenase (Cai *et al*., 2006), resulting in ion imbalance and electrolyte leakage as well as excessive shriveling and softening (Tesfay and Magwaza, 2017; Cheema *et al*., 2018).

Table 6 shows significant results in bitter gourd chemical characteristics such as pH and TA as influenced by the varying illumination. However, the result of this study yielded insignificance in terms of EC, TDS, TSS, and Vitamin C. It agrees with the findings of D'Souza *et al*. (2015) that the white LED (500-700 nm) imposed did not influence the ascorbate production. It can deduced that light treatments with 1.5 W in 4 h have insufficient doses to bring significant improvement in the said metabolite. Exposure to continuous white and blue LEDs resulted in a slight increase in ascorbic acid (Asc) content at the end of broccoli storage (Hasperué *et al*., 2016). Sweet peppers with Blue light at 450 nm in 8 h per day had minimum changes in ascorbic acid content (Thilini Deepashika Perera *et al*., 2022). Blue LED at a lower intensity, 20 µmol m^{-2} s^{-1} under 5°C, decreased the Asc content of broccoli while maintaining the levels at a higher intensity, 50 µmol m^{-2} s^{-1} (Loi *et al.*, 2019). Red, blue, and green lights at 20, 40, and 60 W m^{-2} increased the TSS of

Table 6 - Chemical parameters of bittergourd as influenced by illumination colors during storage. Data represent mean ± deviation standard

Treatments	Chemical parameters					
	pH value	EC mS	TDS ppm	TSS °brix	TA %	Vit. C mg 100 g^{-1}
Control (No LEDs)	7.83±0.06 a	3.12±0.25 NS	2153.33±142.24 NS	1.83±0.14 NS	0.49±0.11 b	0.14±0.02 NS
White LEDs	7.73±0.12 a	3.26±0.55 NS	2233.33±390.68 NS	1.92±0.14 NS	0.38±0.08 b	0.12±0.04 NS
Blue LEDs	7.16±0.12 b	3.05±0.18 NS	2086.67±128.97 NS	1.83±0.14 NS	0.47±0.14 b	0.18±0.03 NS
Red LEDs	7.16±0.06 b	2.94±0.53 NS	2013.33±355.29 NS	1.75±0.25 NS	0.47±0.07 b	0.19±0.05 NS
Initial data	4.60±0.00 c	2.40±0.00 NS	1640.00±0.00 NS	2.00±0.00 NS	0.86±0.00 a	0.18±0.00 NS
CV (%)	1.18	12.49	12.41	8.47	17.34	19.98

Data represent mean ± deviation standard.
Mean values (n = 5) from three replicates in each column of sampling times followed by different letters are significantly different from each other at 5% Tukey's test; NS= not significant.
CV= Coefficient of variation.

fruits (Nassarawa *et al.*, 2020). A single application of RL to harvested vegetables increased the sugar, soluble protein, and vitamin C content (Loi *et al.*, 2020; Poonia *et al.*, 2022). Bitter gourd has a low TSS (°brix) compared to other fruits with high sugar content, such as pitaya, which contains several monosaccharides that contribute to sweetness. In addition, the sugar content of the skin of grape berries was enhanced after red and blue light exposure at 50 µmol m^{-2} s^{-1} (D'Souza *et al.*, 2015). Cell wall breakdown and postharvest decay were delayed by blue light emission within 2 h at 25°C, which decreased monosaccharides such as glucuronic acid in pitaya fruits (Pott *et al.*, 2020).

4. Conclusions

The current study aimed to investigate the effects of white, blue and red Light Emitting Diodes (LEDs) on the pigment composition, physical and chemical parameters of bittergourd (*Momordica charantia* L.). Irradiated and non-irradiated fruit samples were at ambient temperature with an 8 h continuous light source from the room ceiling. Based on the present findings, the white and blue LEDs delayed fruit shrivelling and yellowing on the third day and maintained an excellent visual appearance on the fifth day compared to the control and red LEDs. White LEDs had lower weight loss, followed by red and no LEDs on the third day. Among the treatments, the dry matter content decreased from the initial day. The white, blue, and red LEDs did not prevent fruits from yellowing, but there was a slight change compared to the control with the lowest amount of total chlorophyll a and b, whereas the carotenoids remained stable. Regardless of treatments, the pH values (neutral) increased from the initial day (acidic). However, the blue and red LEDs had lower pH values compared to white and control. Irrespective of treatments, the EC, TDS, and vitamin C remained stable during storage. In contrast, TA lowered from the initial day. White and blue reached a shelf-life of 5 d, whereas red LED and control until four days, numerically.

Generally, LEDs with 1.5 W had the potential to improve the quality and shelf-life of bitter gourd fruits. Among the treatments, the white LED positively affected the physical and chemical parameters. Increasing the wattages or light intensities under continuous or intermittent lighting with lower temperatures makes it possible to achieve preserved fruit quality beyond five days of shelf-life. Based on the results, it would suggests potentiality of LEDs for the future studies, which can be a useful information towards preserving the physico-chemical attributes of bittergourd and other postharvest crops.

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The effect of thymol and carvacrol rich-plant essential oils on controlling postharvest decay molds in orange fruit

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Abstract: The antifungal activity of essential oils of *Thymus daenensis*, *Thymus vulgaris*, *Satureja hortensis* and *Satureja khuzistanica* as well as their major compounds were studied against mold decays of orange fruit. According to GC-MS analysis, the major compounds of *T. danensis* essential oil were thymol (65.5%) and alpha-terpinene (11.9%) whereas *T. vulgaris* was rich in thymol (59%) and p-cymene (15.6%). Carvacrol (88.4%) in *S. khuzistanica* oil and carvacrol (51%), gamma-terpinene (20.8%) and p-cymene (13.7%) in *S. hortensis* oil were charecterized as major compounds. The oil of *S. khuzistanica* and its major compound carvacrol exhibited the strongest fungicide activity against *Penicillium digitatum, Rhizopus stolonifer* and *Colletotrichum gloeosporioides* at 300 µL/L. The results on orange fruits exhibited that the use of *S. khuzistanica* and *S. hortensis* EOs as spraying and dipping treatments could considerably reduce spoilages decays in the fruit.

1. Introduction

Post-harvest diseases of fruits are mainly caused by fungal species such as *Botrytis* spp., *Colletotrichum* spp., *Aspergillus* spp., *Alternaria* spp., *Rhizopus* spp. and *Penicillium* spp. (Agrios, 2005). The fruit decay caused by post-harvest diseases is usually more than what is thought, because with the decrease in yield the price of damaged fruits (Wills and Golding, 2016). Citrus fruits, especially oranges, are among the fruits that are highly sensitive to fungal infections. The use of fungicides, such as benomyl, thiabendazole and imazalil, is the most common method of controlling post-harvest decays of citrus fruits. These fungicides have health and environmental problems such as cumulative and carcinogenic properties in living organisms and acute or chronic poisoning effects. In addition, resistance to these fungicides is increasing in the population of pathogens (Sharifi-Tehrani and Farzaneh, 2018). Anyway, the increase in global demand for providing sufficient and healthy food, based on health standards, along with the policies of the World Food and Agriculture

Organization (FAO) and the Environmental Protection Organization (EPO) has caused extensive research to be carried out. According to the Food and Drug Administration (FDA), the essential oils (EOs) of some medicinal plants are known as natural and healthy alternatives to chemical fungicides and are more acceptable to the public (Brun *et al*., 2003; Carvalho de Sousa *et al*., 2004; Nazzaro *et al*., 2017).

EOs are volatile and natural complex compounds that are characterized by their sharp and strong smell and are formed as secondary metabolites in aromatic plants. Some EOs that have antiseptic properties (antibacterial, antiviral and antifungal properties) are used in food and pharmaceutical industries (Burt, 2004; Bolouri *et al*., 2022). In nature, EOs play an important role in protecting plants against bacteria, viruses, fungi and insects (Regnault-Roger *et al*., 2012; Zitzelsberger and Buchbauer, 2015). They may also attract a number of insects to disperse pollen and seeds (Bakkali *et al*., 2008). Medicinal plant EOs not only have no side effects (at the right concentration), but due to their antioxidant properties, may increase the quality and storage time of fruits (Arras and Usai, 2001; Anthony *et al*., 2003; Plotto *et al*., 2003; Plaza *et al*., 2004). Research has shown that aromatic plants belonging to the Lamiaceae and Asteraceae families are rich in antimicrobial and antioxidant compounds (Barroso and Ruberto, 1998; Farzaneh *et al*., 2006 a, b; Farzaneh *et al*., 2015). The antifungal property of EOs is also related to some of their compounds such as carvacrol, menthol, cymene, thymol, cinnamaldehyde, eugenol, pinene, and linalool, which are known as compounds with high antifungal effect (Cimanga *et al*., 2002).

The purpose of this research is to investigate the potential of EOs of plants rich in thymol and carvacrol, such as *Thymus danensis*, *Thymus vulgaris*, *Satureja hortensis*, and *Satureja khuzistanica* in preventing rot and decay of orange fruit caused by *Colletotrichum gloeosporioides*, *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium digitatum*.

2. Materials and Methods

Inoculum preparation of pathogens

Four fungi that cause post-harvest decay of orange fruit, including *C. gloeosporioides*, *A. niger*, *R. stolonifer,* and *P. digitatum*, were obtained from the mycology collection of the Department of Plant protection, Agriculture and Natural Resources Campus, University of Tehran. In order to prepare the pathogen inoculum, 5 mL of distilled sterile water containing 0.05% Tween 80 was added to the seven-day old culture of each fungus on PDA medium and the surface of the colony was scraped to provide spores and mycelia suspension. The resulting suspensions were passed through four-layer cheesecloth, and then the spore population was adjusted to a concentration of $1*10^5$ spores per milliliter using a hemacytometer.

Plant EOs and their major compounds

The aerial parts of tow thyme species, *T. danensis* and, *Thymus vulgaris,* at the flowering stage were collected from Semirom region of Isfahan province, while the aerial parts of two savory species, *S. hortensis* and *S. khuzistanica* were collected from Pol-Dokhtar and Majin regions of Lorestan province, respectively. The collected plant parts were delivered to the Medicinal Plants and Drugs Research Institute (MPDRI), Shahid Beheshti University (SBU) in Tehran. After confirming the identity, the plants were dried at room temperature and shade. Each sample was powdered using a mill, and then their EOs was extracted by distillation with water in a Clevenger according to the method recommended in the British Pharmacopoeia (1988). The standard compounds of thymol, carvacrol, para-cymene and gamma-terpinene were purchased from Sigma-Aldrich Co.

Analysis and identification of EOs compounds

The EO obtained from each plant was identified with gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS) methods. First, one microliter of EO extracted from each plant was injected into the TRACE™ GC 2000 gas chromatograph (ThermoQuest Italia S.p.A., Rodano, Milan, Italy) with a flame ionization detector (FID) and fused silica capillary DB-1 column (60 m × 0.25 mmi.d.; film thickness= 0.25 µm). Injector and detector temperatures were 250˚C and 300˚C, respectively. Helium was used as the carrier gas at a flow rate of 1.1 ml/min; oven temperature was programmed from 60˚C to 250˚C at the rate of 4˚C/min, and finally held isothermally for 10 min. GC-MS analysis was also performed by using a ThermoQuest Finnigan Trace GC/MS (ThermoQuest Italia S.p.A., Rodano, Milan, Italy), equipped with a DB-1 column (60 m × 0.25 mmi.d.; film thickness= 0.25 µm). Gas chromatographic conditions and the thermal programming were as given for GC. Helium was used as carrier gas

with ionization voltage of 70 ev. Ion source and interface temperatures were 200˚C and 250˚C, respectively. Mass range was from *m/z* 43-456. Identification of individual compounds was done by comparison of their mass spectra with those of similar compounds from a database (Wiley/NBS library) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature. The percentage of each compound was determined according to its relative area percentages obtained by FID, without using correction factors (Adams, 2007).

In vitro antifungal assay

The main ingredients of EOs, including thymol, carvacrol, paracymene and gamma terpinene, were obtained from the Phytochemistry Department of MPDRI. Antifungal effect of EOs and main compounds was investigated against four post-harvest decay fungi of fruit by mixing EO with PDA solid culture medium (Farzaneh *et al*., 2006 b). In short, Petri dishes containing concentrations of 75, 150, 300, 600 and 1200 microliters of EO/standard major -compound per liter of culture medium were prepared and after placing a fungal disk (with a diameter of 5 mm) in center of Petri dishes, they were kept at a temperature of 25 °C in darkness. The growth of each fungus colony was measured daily until the surface of control Petri dishes was completely occupied by the fungus. The percentage of growth inhibition was calculated. The minimum inhibitory concentration (MIC) of the EOs was calculated to prevent the growth of fungi. To investigate whether the EO shows fungicidal or fungistatic activity, the fungal disk of the treatments without fungal growth, was re-cultured on the PDA culture medium, and the growth or not growth of the fungus on the PDA was investigated after one week to calculate the minimum fungicidal concentration (MFC). In addition, the EC_{50} value (effective concentration causing 50% inhibition of mycelial growth) was calculated from the data by probit analysis.

Antifungal assay on orange fruit

The healthy orange fruits (Thomson cultivar) free of any chemical and physiological treatment and same in size and ripeness index were provided from Citrus and Subtropical Fruits Research Center, Ramsar, Mazandaran province, Iran. After disinfecting the fruits surface by 70% ethanol for one minute, a wound of 1 mm in diameter and 2 mm in depth (limited to the albedo part in the equatorial region of the fruit) was created on each fruit in sterile condition. Then the fruits were treated with the concentration of 1/1000 (1000 ppm) EO by two methods; dipping and spraying. Then treated fruits were inoculated by spraying of a suspension of 1×10^5 spores per milliliter. Control treatments included dipping and spraying of fruits with Tween 80 solution (0.05%) and thiabendazole fungicide. In this experiment, each treatment contained of 4 replicates and each replicate consisted of 8 experimental units (fruits). The surface of treated fruits was dried under air flow for 2 h and then they arranged on special fiber plates before transferring to a storage room of 25°C and darkness. After the storage period (10 days), the diameter of the decay area on orange fruit was measured using a caliper. The efficacy of the EOs was determined by the formula: IP =(C–T/C)*100), where IP is the inhibitory percentage of the of spoilage decay, and C and T are the spoilage decay area in control and treatment, respectively.

Statistical analysis

To analyze the data, three software were used. At first, the normality of the data was normalized by Mini-tab software Version 17.1 (Minitab Inc. state college, PA). Then SAS software Version 9.1.3 (SAS Institute Inc., Cary, NC) with GLM method was used for variance analysis. After analysis the variance, the mean of the data was compared using Duncan's multi-range test at the 5% level. The EC_{50} values were calculated from the data subjected to probit analysis using IBM SPSS statistics Version 26 (IBM Corp. Chicago, IL).

3. Results

The main compounds of EOs

The main compounds in *T. danensis* EO included thymol (65.5%), alpha-terpinene (11.9%) and paracymene (7.5%) (Table 1). Thymol (59%), paracymene (15.6%) and gamma-terpinene (4.2%) were the main compounds identified in the EO of *T. vulgaris* (Table 1). The main compounds in the EO of the *S. khuzistanica* included carvacrol (88.4%), para-cymene (3%) and gamma-terpinene (4.5%). Carvacrol (51%), gamma-terpinene (20.8%) and para-cymene (13.7%) were the main compounds identified in the EO of the *S. hortensis* species (Table 2).

Table 1 - The major constituents (%) of chemical composition of *Thymus daenensis* and *T. vulgaris* essential oils

No.	Compound	Retention indices	*T. daenenis* (%)	*T. vulgaris* (%)
1	Alpha-thujene	925	0.8	1.8
2	Alpha-pinene	933	1.6	1.6
3	Beta-pinene	974	0.7	2.6
4	Myrcene	981	1.1	1.9
5	Alpha-phelandrene	999	0.2	1.7
6	Para-cymene	1014	7.5	15.6
7	Gamma-terpinene	1053	-	4.2
8	Alpha-terpinene	1080	11.9	-
9	Thymol	1266	65.5	59.0
10	Carvacrol	1282	0.1	3.1
11	Carvacryl acetate	1345	2.5	2.0
12	Beta-caryophyllene	1424	3.8	1.5
13	Beta-bisabolene	1501	1.3	0.9
Total		-	97.0	95.9

* Retention indices relative to C6-C24 n-alkanes on the DB-1 column.

Table 2 - The major constituents (%) of the chemical composition of *Satureja khuzistanica* and *Satureja hortensis* essential oils

No.	Compound	Retention indices	S. Khuzistanica	S. Hortensis (%)
1	Alpha-thujene	925	-	2.5
2	Alpha-pinene	933	-	2.9
3	Beta-pinene	974	0.2	1.1
4	Myrcene	981	0.2	1.5
5	Para-cymene	1014	3.0	13.7
6	1.8-cineole	1023	0.7	1.0
7	Gamma-terpinene	1053	4.5	20.8
8	Carvacrol	1282	88.4	51.0
9	Carvacryl acetate	1345	0.1	1.3
Total		-	97.1	96.7

* Retention indices relative to C6-C24 n-alkanes on the DB-1 column.

Antifungal effect of EOs

The results of the antifungal effect of the EOs on the growth of fungi are shown in Table 3. In general, the more the concentration of EO increased, the more antifungal activity was seen. In addition, the intensity of the EOs inhibitory effects against *C. gloeosporioides* and *R. stolonifer* was more evident (Table 3).

According to the results (Table 3), for controlling the *A. niger* growth, only the EO of *S. khuzistanica* showed the highest antifungal activity with the MIC 300 µL/L. To inhibit the growth of *P. digitatum,* all EOs showed significant antifungal activity with the

Table 3 - The inhibitory activity (%) of four plant essential oils at different concentrations against spoilage fungi of citrus fruit by poisonous PDA medium method

Essential oil	concentration (µl/l)	Inhibitory activity (%)			
		A. niger	*P. digitatum*	*C. gloeosporioides*	*R. stolonifer*
T. daenensis	75	22.16	34.20	11.13	24.96
	150	57.35	56.33	34.20	96.05
	300	90.11	85.71	100	100
	600	100	100	100	100
	1200	100	100	100	100
T. vulgaris	75	20.94	27.11	3.38	0.60
	150	65.45	55.55	31.77	90.22
	300	75.01	73.34	82.29	100
	600	100	100	100	100
	1200	100	100	100	100
S. hortensis	75	16.22	28.92	2.49	14.25
	150	27.94	39.11	58.33	51.55
	300	88.32	78.92	100	100
	600	100	100	100	100
	1200	100	100	100	100
S. khuzistanica	75	24.10	11.50	26.32	25.81
	150	67.91	60.00	80.77	96.26
	300	100	90.66	100	100
	600	100	100	100	100
	1200	100	100	100	100

MIC 600 µL/L., whereas the EOs of three species, including *S. hortensis*, *S. khuzistanica* and *T. danensis* showed the great antifungal activity against *C. gloeosporioides* with the MIC 300 µL/L. To inhibit *R. stolonifer*, all four EOs with the MIC 300 µL/L showed the noticeable antifungal activity.

The results (Table 4) obtained from the re-culture of fungal disks, in the treatments which no fungal growth was observed, showed that none of the EOs had fungicide properties on the *A. niger*. Two EOs of *S. khuzistanica* and *S. hortensis* at a concentration of 1200 µL/L showed fungicidal properties against *P. digitatum,* whereas *T. daenensis* and *T. vulgaris* EOs showed the MFC values more than 1200 µL/L.

Essential oil of *S. khuzistanica* showed MFC against *C. gloeosporioides* at MFC 600 µL/L, while *S. hortensis* and *T. danensis* EOs exhibited MFC of 1200 µL/L of culture medium. Howevere, *T. daenensis* oil didn't show MFC value at the maximum concentration. To control of *R. stolonifer*, the EOs of *S. khuzistanica* and *S. hortensis* showed MFC at the concentrations 300 µL/L and 600 µL/L, respectively, while both *T. vulgaris* and *T. danensis* EOs exhibited MFC at the concentration of 1200 µL/L (Table 4).

Table 4 - Minimum fungicidal concentration (µl/l) of four essential oil against citrus fruit spoilage fungi. The experiments were carried out *in vitro* by Poisonous PDA Medium method

Fungi	*S. khusiztanica*	*S. hortensis*	*T. danensis*	*T. vulgaris*
A. niger	>1200	>1200	>1200	>1200
P. digitatum	1200	1200	>1200	>1200
C. gloeosporioides	600	1200	1200	>1200
R. stolonifer	300	600	1200	1200

Antifungal properties of the main components of EOs

In general, by increasing the concentration of the EO/standard main-component its antifungal activity increased (Table 5).

Among the main compounds, carvacrol exhibited the highest antifungal activity. The MIC of carvacrol against the growth of *R. stolonifer* was 150 µL/L. Carvacrol at a concentration of 300 µL/L prevented the growth of other fungi as well. Thymol was another main compound in the EOs, especially thyme, which showed considerable antifungal activity. Thymol at the MIC of 300 µL/L completely prevents

Table 5 - The inhibitory activity (%) of four major compounds of essential oils at different concentrations against spoilage fungi of citrus fruit by poisonous PDA medium method. The percentage of inhibition in each treatment corresponds to 4 repetitions (4 Petri dishes with a diameter of 8 cm)

Compound	Concentration (µl/l)	Inhibitory activity (%)			
		A. niger	*P. digitatum*	*C. gloeosporioides*	*R. stolonifer*
Thymol	75	10.20	6.70	14.65	14.50
	150	55.48	55.75	66.30	58.86
	300	95.34	100	100	100
	600	100	100	100	100
	1200	100	100	100	100
Carvacrol	75	22.71	29.63	31.88	36.40
	150	64.95	65.50	88.84	100
	300	100	100	100	100
	600	100	100	100	100
	1200	100	100	100	100
Para-cymene	75	0	0	0	0
	150	30.87	28.44	36.50	33.96
	300	61.54	55.89	69.74	62.94
	600	93.38	94.61	100	100
	1200	100	100	100	100
Gamma-terpinene	75	0	0	0	4.69
	150	19.85	15.32	26.58	27.63
	300	63.35	88.84	89.12	85.38
	600	96.48	100	100	100
	1200	100	100	100	100

the growth of three fungi; *C. gloeosporioides*, *R. stolonifera,* and *P. digitatum* whereas the growth rate of *A. niger* was inhibited by 95.3%. Para-cymene had also showed antifungal activity that was able to inhibit the growth of all the fungi at the concentration of 1200 µL/L. Among the fungi, *R. stolonifer* and *C. gloeosporioides* were more sensitive to para-cymene and their growth was completely inhibited at the concentration of 600 µL/L. It is also necessary to mention that this compound did not show any significant antifungal effect against any of the fungi at the low concentrations (<150 µL/L). Gamma-terpinen is one of the main components of EOs, especially in the savory plants that at the MIC concentration of 600 µL/L caused a complete inhibition of the growth of all fungi except *A. niger.* In the other hand, the fungus *A. niger* was the most resistant fungus to this compound, whose MIC was 1200 µL/L. Low concentrations of this compound did not show the inhibitory effect on the growth of the fungi (Table 5).

The results of the MFC indicated that gamma terpinene at any of the concentrations did not cause the death of the fungi. In addition, carvacrol and thymol showed strongest fungicidal activity with MFC 600 µl/l against *R. stolonifer*. Carvacrol also exhibited strong fungicidal activity (MFC 600 µl/l) against *C. gloeosporioides.* However, *A. niger* had the highest resistance to the compounds, and its MFC value was often more than 1200 µl/l (Table 6).

In addition, the antifungal potency of each EO and its main compound was determined according to EC_{50} value as well (Table 7). The lower the EC_{50} indicates the less the concentration of antifungal compound that is required to inhibit 50% of fungal growth. In general, the lowest EC_{50} values were achieved by *S. khuzistanica* EO that showed EC_{50} values of 95.14, 108.00, and 120.93 µL/L against *R. stolonifer, C. gloeosporioides*, and *A. niger*, respectively. In confirmation of it, carvacrol showed the lowest EC_{50} values of 80-124 µL/L against four citrus fruit spoilage fungi.

Table 6 - Minimum Fungicidal Concentration (µl/l) of major compounds of essential oils; thymol, carvacrol, para-cymene and gamma-terpinene; against spoilage fungi of citrus fruit. The experiments were carried out *in vitro* by poisonous PDA medium method

Fungi	Thymol	Carvacrol	Para-cymene	Gamma-terpinene
A. niger	>1200	>1200	>1200	>1200
P. digitatum	1200	1200	>1200	>1200
C. gloeosporioides	1200	600	>1200	1200
R. stolonifer	600	600	1200	>1200

Spoilage decay control on fruit

In general, the application of EOs by dipping method showed the greatest effect in reducing spoilage and fruit rot, whereas the spraying method also had significant effect. In addition, *S. khuzistanica* essential oil was the most effective oil to reduce *A. niger* (95.4%) and *P. digitatum* (86.8%) decays area on the fruit in dipping method. The EO of *S. khuzistanica* had the greatest effect against *R. stolonifer* and *C. gloeosporioides* decays on the fruits by both methods of dipping and spraying of the fruit which could completely (100%) inhibit the both decays

Table 7 - The EC_{50} value (effective concentration causing 50% inhibition of mycelial growth) of each essential oil and its major compounds against spoilage fungi of citrus fruit on PDA (µL/L) calculated by probit analysis

Essential oil/compound	*A. niger*	*P. digitatum*	*C. gloeosporioides*	*R. stolonifer*
T. danensis	154.40 (118.69-197.18) [z]	151.86 (97.52-219.75)	164.02 (151.83-178.53)	95.84 (88.81-103.094)
T. vulgaris	172.02 (80.34-324.16)	179.65 (108.88-283.32)	210.16 (194.97-226.88)	119.23 (111.86-125.91)
S. hortensis	191.92 (176.59-209.02)	186.28 (136.57-256.56)	141.95 (134.20-150.60)	143.68 (132.68-156.79)
S. khusiztanica	120.93 (111.04-131.95)	160.21 (118.11-213.54)	108.00 (99.28-117.03)	95.14 (88.11-102.40)
Thymol	156.31 (131.03-187.02)	143.14 (133.79-154.54)	128.59 (119.27-139.13)	135.97(125.77-147.96)
Carvacrol	124.70 (114.56-136.16)	119.49 (109.03-131.12)	97.53 (89.30-105.78)	80.92 (78.78-83.06)
Para-cymene	289.02 (191.82-459.22)	230.03 (171.91-329.54)	296.60 (213.73-432.76)	245.27 (183.04-365.97)
Gamma-terpinene	284.61 (213.66-403.15)	220.44 (206.91-234.68)	207.22 (193.60-221.86)	207.85 (193.04-224.10)

[z] Numbers in parentheses indicate 95% confidence limits determined by probit analysis.

(Table 8). In addition, *S. hortensis* could completely inhibit *R. stolonifera* decay. However, *T. danensis* and *T. vulgaris* couldn't completely inhibit of the any fruit fungal decay and exhibited weak fungicide activity on the fruit. In addition, fungicide tiabendazole could completely control *R. stolonifera* decay. It seems that *P. digitatum* and *A. niger* are the most resistance fungi to these EOs on the orange fruit.

4. Discussion and Conclusions

In our study, all four plants EOs (belong to Thymus and Satureja geniuses, Lamiaceae) exhibited considerable antifungal activity against postharvest spoilage fungi. It has been found that some medicinal plants of the Lamiaceae family have high antifungal properties (Bakkali *et al*., 2008; Adeyinka and Richard, 2015). Thymol was included the main part (more than 50%) of *T. danensis* and *T. vulgaris* EOs whereas S. *khuzistanica and S. hortensis* EOs were rich in carvacrol (more than 50%). In addition, their major compounds and specially thymol and carvacrol resulted in strong fungictatic and fungicide activities. However, the lowest MFC and EC_{50} values were obtained by *S. khuzistanica* oil and carvacrol. The antibacterial and antimicrobial properties of the main components of EOs such as cinnamaldehyde, eugenol, thymol and carvacrol have been identified in several studies (Bakkali *et al*., 2008; Adeyinka and Richard, 2015). The antimicrobial and antifungal activity of the EO may be due to the characteristics of terpenes/terpenoids compounds, which, due to their high lipophilic nature and low molecular weight, that enable them destroying cell membranes, and inhibiting spore germination (Bakkali *et al*., 2008; Nazzaro *et al*., 2017). However, the dominant composition of the EO may cause the antifungal activity of the EO alone or in synergic manner with other compounds (Plotto *et al*., 2003). Therefore, in our study, the antifungal property of these EOs can be contributed to their thymol or carvacrol content, although other EO constitutes may act synergistically and increase the antifungal activity of the main compound. Research has shown that aromatic plants belonging to the families *Lamiaceae* and *Asteraceae* are rich in antimicrobial and antioxidants compounds and increase the quality of the fruit and the length of its storage period as well (Tajkarimi *et al*., 2010; Hyldgaard *et al*., 2012; Gyawali and Ibrahim, 2014). In addition, EOs could control post-harvest diseases due to their antifungal effects on the both vapor and non-vapor phases (Tripathi *et al*., 2008).

In our study, the application of EOs by dipping method showed the more fungicide activity than the spraying method in terms of reducing spoilage and fruit rot. None of the four EOs and their dominant compounds at the maximum concentration studied in this research (1200 µl/l) could completely controlled *A. niger in vitro* and on fruit conditions, which indicates the high tolerance of this fungus to EO compounds. In addition, although both savory oils could completely kill *P. digitatum* by 1200 µl/l *in vitro*, they couldn't completely inhibit the *P. digitatum* decay on fruit. On the other hand, the sensitivity of *P. digitatum* to EO would be reduced on fruit. However, savory oils could completely inhibit *R. stolonifer* and *C. gloeosporioides* decays on fruit.

Although, the significant *in vitro* antifungal activity of the EOs studied in this research depended on the content of carvacrol and thymol, the EOs of both

Table 8 - The control of orange fruit fungal decays by four medicinal plants essential oils (1 per 1000) trough spraying and dipping methods, after 10 days' incubation in the dark condition at 25°C.

Treatment	Disease incidence (%)							
	Spraying method				Dipping method			
	A.n	P.d	C.c	R.s	A.n	P.d	C.c	R.s
T. daenensis	25.8 c*	38.8 b	9.4 ef	11.0 e	16.3 d	17.6 d	4.5 fg	7.2 f
T. vulgaris	25.4 c	33.5 b	13.7 de	12.4 de	16.1 d	25.4 c	5.0 fg	6.6 f
S. hortensis	9.6 ef	27.2 c	0.0 g	3.3 fg	7.2 f	16.3 d	0.0 g	0.0 g
S. khuzistanica	7.2 f	22.0 c	0.0 g	0.0 g	4.5 fg	12.5 de	0.0 g	0.0 g
Tiabendazole	7.6 f	21.5 c	3.8 fg	3.3 fg	3.5 fg	7.8 f	3.3 fg	0.0 g
Infected Control	97.5 a	97.5 a	95.0 a	95.0 a	97.5 a	95.0 a	95.0 a	95.0 a

An= *Aspergilus niger*; Pd= *Penicillium digitatum*; Cc= *Colletotrichum gloeosporioides*; Rs= *Rhizopus stolonifer*.
Means followed by the same letter within a column are not significantly different at *P≤0.05*.

savory species (rich in carvacrol) were more effective than thyme species oils (rich in thymol) in terms of controlling fungal decays on fruit. Finally, plant EOs rich in carvacrol are introduced as promising candidates for the commercial production of natural fungicides to disinfection and management of post-harvest decay molds of citrus fruits.

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Physiological performance and fruit quality of noni (*Morinda citrifolia* L.) cultivated in different agro-climatic zones of Fiji

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Abstract: Noni (*Morinda citrifolia*) fruit juice is widely used as a strong antioxidant nutritional supplement. With its demand for supplementary products globally, commercial noni farming is now increasing in the Pacific Islands. Information on its growth performance and fruit quality under variable climatic condition is limited. This study aimed to establish the climatic requirements and identify the agro-climatic zone in Fiji that provides for increased antioxidant levels in fruits in addition to optimal plant growth and physiological performance. The study investigated plant growth, photosynthetic performance, fruit yield and antioxidant properties of plants that were cultivated under rain fed conditions in the dry, wet and intermediate agro-climatic zones in Fiji Islands. The physiological performance was significantly influenced by the soil moisture, sunshine hours and soil nutrients. Physiological performance including fruit yields were the highest in the intermediate zone which was characterized by a moderate rainfall and fairly good soil properties while it was lowest in the dry zone. Highest fruit antioxidant properties occurred in the dry zone followed by wet zone. The study implies that under cultivation, moderate abiotic stress can enhance the antioxidant properties of noni.

1. Introduction

Noni (*Morinda citrifolia* L.) is a tropical evergreen perennial plant with large elliptical leaves and a compound fruit (Nelson and Elevitch, 2006). Noni is native to is native to Southeast Asia and Australia and has a pantropical distribution (Nelson, 2003; Pandiselvi *et al.*, 2019) The plants are significant source of traditional Polynesian medicine. The fruits range from 4-10 cm in length and about 3-4 cm in diameter and are the most commonly used parts. The fruit has a broad range of nutraceutic and therapeutic potentials (Chan-Blanco *et al.*, 2006; Mahantesh *et al.*, 2018; Almeida *et al.*, 2019; Inada *et al.*, 2020). Noni fruit products have become quite popular in the area of health care due to its biologically active com-

pounds and high antioxidant potential. Commercial noni farming occurs in most tropical countries and is now growing in the Pacific Islands.

Growing noni under rain fed conditions are highly beneficial in tropical countries where rainfall is abundant with good volcanic soil (Nelson and Elevitch, 2006). Most countries, however, have their own agro-climatic zones where certain crops perform well. Agro-climatic zone is the characterization of an area based on its climatic parameters that are suitable for agriculture (Parry *et al.*, 1988). Farming in agro-climatic zones ensures that a crop is ecologically viable together with being economically profitable. Amin *et al.* (2004), lists some agro-climatic parameters as rainfall, maximum and minimum temperature, humidity, evapotranspiration, maximum possible sunshine hours and wind speed. Crop growth and agricultural productivity are primarily affected by all climatic elements, whether its effects occur singly or in combination (Parry *et al.*, 1988; Mittler, 2006). Precipitation, temperature and solar radiation have a direct effect on key plant metabolic processes such as photosynthesis and respiration while humidity is crucial for regulating transpiration and plant water balance. Sub-optimal values of precipitation, temperature, light intensity and relative humidity can result in crop yield reduction and product quality (Ferrante and Mariani, 2018).

Noni is renowned for tolerating a wide range of climatic conditions in its habitats. According to Nelson (2003), noni is found from 1 m to 800 m above sea level growing in a wide range of soils (infertile soils, acidic and alkaline soils) in its natural habitats. The mean annual rainfall range is from 250-4000 mm, and mean annual temperature range is from 20°C to 35°C. Noni plants can survive dry seasons with less than 40 mm of rainfall for at least 3 - 4 months depending on plant size and age and its surrounding temperature and humidity (Nelson, 2003). According to Nelson and Elevitch (2006), in cultivation, about 500-1500 mm annual rainfall that evenly spreads over the year is ideal for obtaining high yields. In wet areas where the annual rainfall is up to 4000 mm/year, the yield of noni is high, but the fruits are usually very watery, less sweet and tend to have much slower and uneven ripening while in drier areas fruits are much sweeter with rapid and even ripening (Nelson and Elevitch, 2006). Under cultivation, noni has been found to be a very low-maintenance plant which requires low irrigation and fertilizer.

Since noni's commercial market firmly bases its adverts on high antioxidant properties, noni growers must ensure that good plant growth and high fruit yield also accompanies fruits with high antioxidant properties. Antioxidants can be enhanced in fruits by changing some cultivation practices once the specific environmental effects on fruits are known (Dumas *et al.*, 2003; Wang, 2006). Major antioxidants, both enzymatic and non-enzymatic protect higher plant cells from oxidative stress damage that usually occurs when the plants undergo environmental stresses. Antioxidant properties of noni are highly associated with the non-enzymatic phenolic compounds (Dussossoy *et al.*, 2011). The non-enzymatic antioxidants in plants are mainly comprised of ascorbic acid, glutathione, α-tocopherol, carotenoids, phenolics, flavonoids, and amino acid cum osmolyte proline. Due to their essential role in protection and development, high levels of non-enzymatic antioxidants are usually expected in plants undergoing adverse environmental stress hence noni's antioxidants may be elevated under stressful conditions.

Growers often make the cultivation environments ideal and stress-free for plants which are likely to lower the antioxidant capacity of the fruits. Hence apart from increasing the plant growth and fruit yield, it is also important to know what kind of environmental conditions would enhance the antioxidant levels in the noni fruits. This study examined the growth and physiological performance of noni plants cultivated in Fiji's three different agro-climatic zones and compared its growth, fruit production and yield together with its antioxidant properties. The study implicates how noni's cultivation environment can be altered to enhance the antioxidant production together with maintaining the overall productivity.

2. Materials and Methods

Plant growth and physiological performance of noni cultivated in dry, intermediate and wet climate zones of Viti Levu, Fiji were studied for two years from January 2016 to January 2018.

Experimental sites

The main centers of dry (Nadi - 17°45'15"S, 177°28'3"E), wet (Suva - 18°14'80" S, 178°44'76''E) and intermediate (Sigatoka - 18°06'05"S, 177°32'13"E) agro-climatic zones on the island of Viti Levu, Fiji were chosen as the experimental sites (Fig. 1).

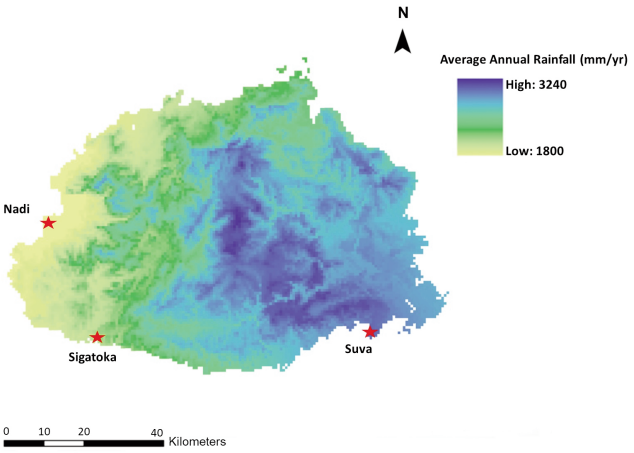

Fig. 1 - Average annual rainfall distribution in Viti Levu and the locations of the study sites in dry zone (Nadi), intermediate zone (Sigatoka) and wet zone (Suva) (Adapted and modified from Printemps, 2008).

Data on rainfall and temperature for 2016 and 2017 was obtained from the Fiji Meteorological Service office for the three sites (Table 1). Soil samples were collected randomly three times during the experiment duration, and the nutrient content was analyzed for the three cultivation sites.

Cultivation of noni

Four-month old noni plants were established in ground plots located in the dry, intermediate and wet agro-climatic zones of the island of Viti Levu, Fiji (Fig. 1). A total of 20 plants were transplanted with a spacing of 2 m. The transplanted plants were watered for the first month on a 3-day interval and on a weekly interval for the second and third month. Starting from the third month of establishment, the plants were left to be rain-fed. Balanced nitrogen, phospho-

Table 1 - Weather and soil attributes of the experimental sites for the two-year growth period (2016 and 2017)

Data	Nadi (Dry zone)	Sigatoka (Intermediate zone)	Suva (Wet zone)
Total rainfall (mm)	2237.3 ± 92.9	1645.5 ± 107.9	3102 ± 299.1
Total rainfall wet months (mm)	1936.2 ± 184.1	1091.7 ± 34.8	2119 ± 128.3
Rainfall dry months (mm)	301.3 ± 90.8	553.5 ± 72.8	982.8 ± 427.4
Mean monthly rainfall (mm)	186.4 ± 41.6 ab	139.3 ± 24.4 b	258.6 ± 37.5 a
Mean rainfall wet months (mm)	322.7 ± 59.5 ab	201.4 ± 38.76 b	353.3 ± 56.55 a
Mean rainfall dry months (mm)	50.2 ± 18.4 a	77.2 ± 16.8 a	163.8 ± 32.3 b
Mean temperature (°C)	26.3 ± 1.4 a	25.8 ± 1.7 a	26.4 ± 1.6 a
Mean max temperature (°C)	30.5 ± 1.2 a	30.7 ± 1.7 b	29.2 ± 1.8 c
Mean Min temperature (°C)	22.0 ± 1.7 a	20.9 ± 1.9 b	23.5 ± 1.4 c
Mean sunshine hours	7.0 ± 0.4 a	5.9 ± 0.4 b	4.9 ± 0.7 c
Mean soil pH	5.7 ± 0.1 a	6.8 ± 0.1 b	7.2 ± 0.1 c
Mean electrical conductivity (mS cm^{-1})	0.01 ± 0 a	0.09 ± 0.01 b	0.27 ± 0.06 c
Mean total nitrogen (N) (%)	0.13 ± 0.03 a	0.19 ± 0.01 b	0.20 ± 0.02 c
Mean available phosphorous (P) (mg/kg)	3.7 ± 0.6 a	36.3 ± 0.6 b	10.6 ± 2.9 c
Mean potassium (K) (mg/kg)	66.5 ± 39.1 a	518.7 ± 19.3 b	520.0 ± 92.2 c
Mean calcium (Ca) (mg/kg)	221.3 ± 23.4 a	5607.3 ± 89.5 b	7568.7 ± 401.1 c
Mean magnesium (Mg) (mg/kg)	35.4 ± 4.4 a	872.7 ± 28.9 b	619.8 ± 7.4 c

Mean ± SE are shown, n=24 (weather attributes) and n= 6 (soil attributes). According to Kruskal-Wallis test at $p<0.05$, there was a significant difference in mean rainfall per month ($p= 0.0332$, $H= 6.810$), rainfall per wet month ($p=0.0664$, $H= 5.423$), rainfall per dry month ($p=0.0030$, $H= 11.63$), maximum temperature ($p=0.0032$, $H=11.50$), minimum temperature ($p= <0.000$, $H= 20.82$), sunshine hours per month ($n=24$, $p=<0.0001$, $H=23.63$). There is no significant difference in monthly temperature ($p =0.4951$, $H= 1.406$). Soil attributes were also significantly different using Kruskal-Wallis test at $p<0.05$, soil pH ($p<0.0001$, $H=15.51$), electrical conductivity ($p=<0.0001$, $H=16.11$), total nitrogen ($p=0.0001$, $H=12.48$), phosphorus ($p=<0.0001$, $H=15.3$), potassium ($p=0.0004$, $H= 11.69$), calcium ($p=<0.0001$, $H= 15.25$) and magnesium ($p<0.0001$, $H=15.25$). Means not sharing the same letter are significantly different using the Mann-Whitney test at $p<0.05$.

Note: Ideal ranges for Fiji soil mineral nutrient content include 0.3-0.6% nitrogen, 20-30 mg/kg phosphorous, 117-234 mg/kg potassium, 400-2000 mg/kg calcium, 122-366 mg/kg magnesium. Source: Koronivia Research Station, Fiji.

rus and potassium (N.P.K) fertilizer was applied in equal amounts (2 g) to each of the plants at the end of the 2-month period. After that, N.P.K fertilizer in a 13:13:21 ratio was applied in equal amounts (2 g) at three months' interval up to 8 months (the end of 5 months and 8 months). Fertilizer treatment was then stopped, and the plants were left to grow in its cultivated environment.

Determination of plant survival and growth rate

Plant survival rates were calculated by counting the number of noni plants that had survived until the end of the two-year period divided by the number of plants that initially planted (20 plants). The growth rates of plants at the three sites were determined using the plant height. Initial plant height was measured on the day of transplanting, and the final height was measured at the end of the experiment. The plant growth rate in cm per day was calculated by dividing the change in height by the number of days.

Gas exchange measurements

Photosynthesis rate (A_n), transpiration (E) and stomatal conductance (g_s) were measured using the portable photosynthesis meter (LCpro-SD by ADC Bioscientific) connected to a broad leaf chamber. At the cultivation plot, mature leaf (fifth fully expanded leaf) from each plant was selected for gas exchange and transpiration measurements. Over the two-year period, gas exchange measurements were done 17 times at random intervals starting from the 5th month of growth at the dry, intermediate and wet sites. The instantaneous leaf water use efficiency (WUE_i) was calculated as the A_n/E ratio.

Determination of changes in fruiting, fruit yield and total soluble solids

The plants started flowering and fruiting by eighth month of growth. Once all plants at the three sites had fully developed fruits, number of fruits (both young and mature) on each plant were counted randomly on eight occasions. Any mature fruit (hard and whitish in colour) present at the time of observation was collected weighed using a top pan balance. The weight of the fruit was recorded as yield in grams. The total soluble solids (TSS) in °Brix was measured using the hand-held refractometer (ATAGO). A total of 10 fruits were randomly collected and sampled.

Determination of antioxidant capacity of fruits

The total antioxidant activity of fruits from different locations was estimated by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method, as described by Yang *et al.* (2011). A total of 10 fruits from each site were used to find out antioxidant capacity where 1 fruit was used as an individual sample. A solution of DPPH was made in methanol using 0.025 g DPPH in 1 L of methanol. Noni fruit was ground, and its juice was extracted using a muslin cloth. Diluted noni fruit extracts (2 µL, 5 µL, 10 µL, 20 µL, 30 µL and 40 µL) were added to the 3 mL DPPH solution and incubated at room temperature for about 40 minutes. After 40 minutes, the absorbance of the mixture was measured at 515 nm with a spectrophotometer (CE1021 UV-VIS). Inhibitions of DPPH radicals in percentage were calculated as follows:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where: $A_{control}$ is the absorbance value of the control reaction (containing all reagents except for the tested fruit extracts) and A_{sample} is the absorbance value of fruit extracts.

The 50% radical scavenging activity was determined by calculating the half-maximal inhibitory concentration (IC_{50}). The IC_{50} value was calculated by plotting the percentage inhibition against the concentrations of fruit extracts. The concentration that provided 50% inhibition was noted as the IC_{50} value. The noni fruit extract concentration at IC_{50} was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg/ 100 g fresh weight (FW) of fruits. To find out the AEAC, a standard curve was prepared using ascorbic acid at 1mg/mL concentration at various concentrations of 2 µg/mL, 3 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL. The antioxidant activity of noni fruits was expressed as AEAC per 100 g of fresh weight (AEAC/100 g FW):

$$AEAC = IC_{50} \text{ (ascorbic acid)}/ IC_{50} \text{ (sample)} \times 10^5$$ (Velde *et al.*, 2013)

Determination of total phenol content in fruits

Total phenol content (TPC) of noni fruits were determined with Folin-Ciocalteu reagent as described by Yang *et al.* (2011). From each site, 10 ripe fruits were collected, ground, and the juice was extracted using a muslin cloth. For each individual sample, 1 fruit was used. Exactly 20 µl of noni fruit extract was mixed with 1.58 mL of distilled water. To this mixture, 100 µl of Folin-Ciocalteu reagent and 300 µl of 20% Na_2CO_3 was added. The mixture was incubated

at 40°C for 30 minutes. After 30 minutes, the absorbance was measured at 765 nm with the spectrophotometer. A standard curve of total phenols was prepared using gallic acid at various concentrations (1 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, 7 mg/mL, 10 mg/mL and 20 mg/mL). The equation of the standard curve was used to determine the TPC and it was expressed as mg of gallic acid equivalent (GAE) per 100 g of fresh weight (mg GAE/100 g of FW).

Statistical analysis

Analysis of data collected was performed by using Graph Pad Prism version 7.0 (GraphPad Software Inc, San Diego, California, USA). Shapiro-Wilk test was used to determine the normality of the data. The assumption of homogeneity of variances was tested using Bartlett's test and Brown-Forsythe test at 95% significance level. One-way ANOVA and Kruskal-Wallis test at 95% significance level were used to test the significant differences among the results obtained for the three sites. Significant differences among the treatments were compared using Tukey's test and Mann-Whitney test at $p<0.05$.

3. Results

Weather and soil attributes of dry, intermediate and wet cultivation sites

The total amount of rainfall for the two-year period was highest for Suva (wet zone) and lowest for Sigatoka (intermediate zone) (Table 1). A distinct difference in rainfall was observed during the dry months (May to October). Highest rainfall level was recorded for Suva area and the lowest level was for Nadi area while Sigatoka area received rainfall in moderate amounts (Table 1). The average temperature and humidity were comparable for the three sites during the two-year study period. Considerable differences were seen in the average sunshine hours for the three sites. Lowest sunshine hours were recorded for the wet zone, followed by the intermediate zone while highest sunshine hours occurred in the dry zone. Considerable differences were also observed for the soil quality among the three sites (Table 1). Soil moisture was highest in the wet site, while it was lowest for the dry site ranging. The wet site had the highest average soil pH and highest EC while the dry site had the lowest. Major mineral nutrition content levels were also different for the three sites. The dry zone site had considerably low nutrient content, average soil total N, available P and K were lowest. For the intermediate site, nutrient levels were adequate. For the wet zone site, N and K levels were adequate. However, available P, however, was quite low.

Plant growth

Survival rate was high in the wet zone and the intermediate zone while it was lowest in the dry zone (Table 2). Decrease in survival rate was due to death of plants that occurred by the end of 9 months. Plant growth rate was highest in the intermediate zone and lowest in the dry zone (Table 2).

Table 2 - The survivability and growth attributes in noni plants in the dry, intermediate and wet cultivation zone

Cultivation zones studied	Survival rate (%)	Growth rate (cm/day)
Dry	45%	0.09 ± 0.009 a
Intermediate	85%	0.23 ± 0.006 b
Wet	80%	0.15 ± 0.011 c

Mean ± SE are shown, n=9 (dry zone), n=15 (intermediate and wet zones). Mean growth rate is significantly different (Kruskal-Wallis test ($P<0.0001$). For the given cultivation zones, means not sharing the same letter are significantly different using the Mann-Whitney test.

Gas exchange attributes

Net photosynthesis (A_n), transpiration (E), instantaneous water use efficiency (WUEi) and stomatal conductance (g_s) (Fig. 2) were significantly different among the cultivation zone. All three physiological parameters were significantly higher in plants grown in intermediate and wet zones compared to the plants that grew in the dry zone. Dry zone plants had the lowest mean A_n (Fig. 2A) while plants in the intermediate zone had the highest A_n. Wet zone plants had comparable mean A_n rate to intermediate zone. Similar patterns were also observed for E, g_s and WUE_i. Mean E recorded was highest for plants growing in the wet zone (Fig. 2B). Plants in the dry area had the lowest mean E. For plants growing in the intermediate E was comparable to the wet zone. Mean g_s of plants was also significantly different among the three zones (Fig. 2D). Lowest mean g_s was recorded for the plants in the dry zone while plants in the intermediate and wet zone plants had comparable mean g_s. WUE_i was also lowest for plants in the

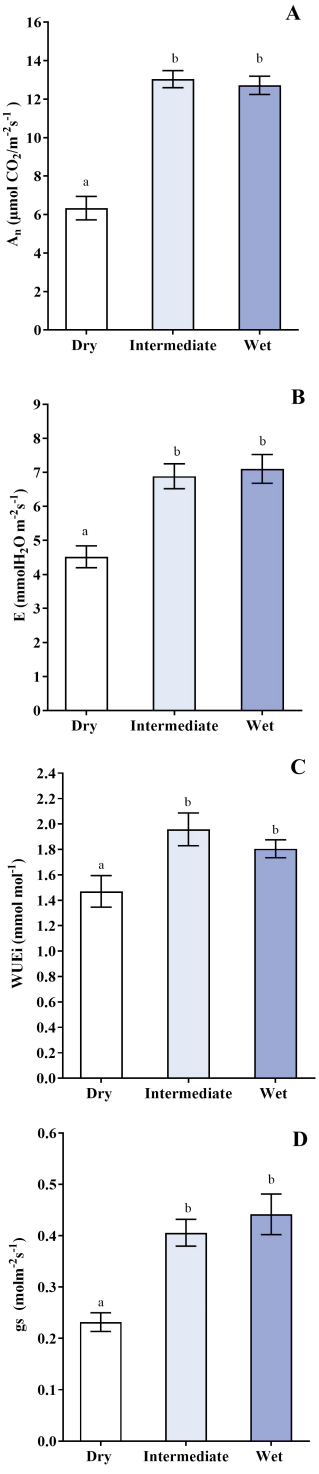

Fig. 2 - Net photosynthesis rate (A), transpiration rate (B), water use efficiency (C) and stomatal conductance (D) of noni plants growing in dry, intermediate and wet cultivation zones. Mean ± SE is shown, n=17. There is a significant difference in An ANOVA (F df (2, 48) = 53.95) p<0.0001, E ANOVA (F df (2, 48) = 14.77) p<0.0001, WUEi ANOVA (F df (2, 45) = 5.05) p<0.0104 and gs ANOVA (F df (2, 48) = 5.807) p<0.0001. For given cultivation zones, means not sharing the same letters are significantly different using Tukey's test.

dry zone (Fig. 2C). Plants in the intermediate zone had the highest WUE_i.

Fruit attributes

Fruit numbers were comparable in the intermediate and wet zone while plants in the dry zone had significantly lower number of fruits (Table 3). Fruit weights were also comparable in wet and intermediate zone while it was lowest in the dry zone (Table 1). Fruit TSS was significantly higher in the dry zone when compared to intermediate and wet zone (Table 3). There was no significant difference in the fruit TSS between intermediate and wet zones.

Table 3 - Mean number, weight and total soluble solids (TSS) of fruits from noni plants in dry, intermediate and wet cultivation zones

Cultivation zones studied	Fruit numbers per plant	Fruit weight (g)	TSS °Brix
Dry	3 ± 0.25 a	74.7 ± 3.8 a	8.4 ± 0.1 a
Intermediate	8 ± 0.28 b	299.8 ± 41.5	7.9 ± 0.1 b
Wet	7 ± 0.30 b	271 ± 19.7 b	7.7 ± 0.1 b

Mean ± SE is shown, n=9 (dry zone), n=15 (intermediate and dry zones). There is significant difference in fruit numbers per plant ANOVA (F df (2, 33) = 0.6480, (p<0.0001), fruit weight ANOVA (F df (2, 42) = 11.75, (p<0.0001) and fruit sweetness ANOVA (F df (2, 36) = 0.7563, (p<0.0001). For given cultivation zones, means not sharing the same letters are significantly different using Tukey's test.

Fruit antioxidant properties

Antioxidant activity (AEAC) in fruits was significantly higher in dry and wet zones compared to the intermediate zone (Table 4). AEAC was comparable for dry and wet zones AEAC while it lowest in the intermediate zone. There was with no significant difference in the mean IC_{50} value between the wet and the dry zone as well (Table 4). TPC in fruits was significantly different at the three sites (Table 4). Highest mean TPC was obtained for fruits in dry location followed by fruits in the wet zone. TPC was lowest for fruits from the intermediate zone.

4. Discussion and Conclusions

When taking into consideration the climate (rainfall, temperature, humidity and sunshine hours) and the soil features over the two-year experimental period for the three different sites (Table 1), differ-

Table 4 - Total phenol content (TPC), radical scavenging activity (IC50) and antioxidant activity (AEAC) in fruits from dry, intermediate and wet cultivation zones

Cultivation zones studied	Fruit numbers per plant	Fruit weight (g)	TSS °Brix
Dry	3 ± 0.25 a	74.7 ± 3.8 a	8.4 ± 0.1 a
Intermediate	8 ± 0.28 b	299.8 ± 41.5	7.9 ± 0.1 b
Wet	7 ± 0.30 b	271 ± 19.7 b	7.7 ± 0.1 b

Mean ± SE is shown, n = 10. There was a significant difference in TPC ANOVA (F df (2, 21) = 0.2139, (p<0.0001), IC50 values ANOVA (F df (2, 21) = 3.760, (p<0.0001) and AEAC content ANOVA (F df (2, 21) = 17.58, (p<0.0001). For given cultivation zones, means not sharing the same letters are significantly different using Tukey's test.

ences in plant growth rates and the physiological performance is clearly due to the differences in water and nutrient availability. Water availability, together with most required essential nutrients such as N, K, Ca, and Mg was sufficient in the intermediate and wet zones compared to the dry zone.

Highest yielding plants that also maintained higher photosynthesis, transpiration and water use efficiency occurred at the intermediate zone. In comparison to dry and wet zones, this cultivation zone had moderate amount of rainfall and good soil quality overall. Highest plant growth rate (Table 2), high gas exchange (Fig. 2) and high fruit yields (Table 3) in the intermediate zone showed that water availability and nutrients were not an issue for this zone. Even though the annual total rainfall in this zone was less than the dry zone, the dry months (May to October) here had considerably higher rainfall compared to the dry zone and lower rainfall compared to wet zone (Table 1). During the dry months, the zone's total precipitation was 553.5 mm which falls in the range required to grow high yielding noni plants. Nelson and Elevitch (2006), also stated that for high yields, noni cultivation site should receive moderate rainfall preferably 500-1500 mm annually that is distributed evenly throughout the year. Higher growth in the intermediate zone was complemented by the high mineral nutrient content of the soil. Major essential mineral nutrients required for plant growth such as N, K, Ca, Mg including P levels were considerably higher than their ideal range in the soil at this site (Table 1). Water availability was highest in the wet cultivation zone together with adequate soil nutrients (Table 1). This site had the highest mean rainfall within the two-year period (Table 1). The soil N and K content were comparable to the intermediate zone. Ca, and Mg were considerably higher in this zone (Table 1). P content, however, was lowest in the wet zone. With generally good soil properties plus plentiful of water, the growth and physiological performance of noni in the wet zone were expected to be much higher. Sunshine hours of 4.9 hours (Table 1) in the wet zone was lowest compared to the other zones as the area had cloudy and rainy days more often. Similarly, transpiration rates and WUE_i were also comparable between the two zones. One crucial factor that may have influenced the physiological performance of noni plants in this zone by limiting photosynthetic activity is the much lower soil P content (Table 1). Short-term inadequate supply of P limits the photosynthesis rates due to restriction of photophosphorylation during the light reactions (Rychter and Rao, 2005). However, the effect of low P content on the physiology of noni is not that strongly evident. Phosphorous deficiency symptoms were not seen on the leaves as well. Noni may have some adaptive mechanism for surviving in P limited soils. Mo *et al.* (2019), showed that some tropical lowland forest plants are able to acclimatize to low P levels by changing the foliar P allocation to fulfil the P demand for photosynthesis. Acclimatization of noni plants to low phosphorous levels requires further investigation. The dry cultivation zone had significantly lower water and nutrient availability (Table 1). The combined effects of water deficit and nutrient deficiency stress lowered the growth of noni in the dry zone. Low photosynthesis and transpiration rates, together with low WUEi of plants (Fig. 2) in this agro-climatic zone can also be attributed to both water and nutrient deficiency. The climatic conditions on days of measurement, especially during the dry season were optimal (i.e. fine sunny days) for high transpiration rates at the dry site, but the rates were significantly low. This was mainly due to partial stomatal closure as indicated by the low stomatal conductance (Fig. 2D). Low stomatal conductance indicates a low degree of stomatal opening, resulting in low rates of incoming CO_2 and outgoing water vapour. The stomatal conductance is usually higher in K deficient plants (as was the case here) under drought stress since K deficiency impairs stomatal function by signalling for ethylene production which in turn inhibits the action of abscisic (ABA) on stomata delaying the closure (Wang *et al.*, 2013). However, low K levels in the dry zone did not appear to influence the stomatal closure. During drought stress in a drought-resistant

plant, ABA level initially increases leading to stomatal closure, but as the drought stress is continued, ABA levels decrease markedly, and stomatal closure becomes water potential-driven (Brodribb and McAdam, 2013). This strategy allows the plants to respond to any rainfall quickly and open the stomata much faster for gas exchange than a non-drought tolerant plant. Drought tolerant plants are also able to maintain gas exchange to gain carbon for a longer period of time during drought (Tardieu and Davies, 1993). For drought tolerant plants, complete stomatal closure is avoided or delayed during drought stress, this helps to maintain the carbon balance (Brodribb and McAdam, 2013). This appeared to be the case for noni plants growing in the dry zone as the stomatal conductance measurements of noni leaves over the two-year period did not show complete closing of stomata in the dry zone. Noni has also been claimed to be a drought-tolerant plant (Nelson, 2003; Singh and Rai, 2007).

Being drought-tolerant, WUE_i in noni plants from the dry area was also expected to be comparable to wet and intermediate zone plants. However, the WUE_i was significantly lower in the dry zone (Fig. 2C) indicating that carbon gain per water loss was low, which resulted due to the low photosynthesis rates and subsequently smaller plants. Nutrient deficiency stress being an add-on to water stress in the dry zone limited photosynthesis activity leading to low WUE_i, hence overall productivity of the plant was low. A decrease in photosynthesis was also due to low amounts of CO_2 entering the leaves as a result of low stomatal conductance. In addition, an increase in leaf temperature may have enhanced the suppression of photosynthesis in the dry zone. According to Haldimann *et al.* (2008), reduced stomatal conductance and reduced transpiration rate raise leaf temperature by several degrees resulting in suppression of photosynthesis due to reversible inactivation of Rubisco. There was no considerable difference between the mean air temperature and the maximum air temperature between the three zones (Table 1). However, an increase in leaf temperature in the dry zone may have resulted from significantly longer sunshine hours (Table 1). Marias *et al.* (2017), showed that an increase in leaf temperature due to reduced stomatal conductance could be very dramatic in the full sun compared to partial sun.

High fruit yield in the intermediate zone (Table 3) also showed that climate and soil features of the intermediate zone were ideal for growing noni under rain fed conditions. Effects of water stress on fruit production and yield is highly evident as seen from the lowest yield in the dry zone. Similar results under drought conditions have been reported for apples (Yao *et al.*, 2001), peach (Rahmati *et al.*, 2018), citrus (Huang *et al.*, 2000), oranges (Perez-Perez *et al.*, 2009) and tomatoes (Sivakumar and Srividhya, 2016. According to Rahmati *et al.* (2018), fruit sizes during drought stress decrease because of reduced water flow to the fruits due to fruit stomatal closure, development of thick cuticles or due to reduction in microcrack occurrences. Nutrient deficiency can also be a factor contributing to fruit size as fruit size is also depended on the number of cells at anthesis (Bohner and Bangerth, 1988). Ca and K, which have crucial roles in cell division and stomatal conductance respectively are lower in the dry zone leading to smaller fruit size. Despite being smaller in size and producing a low number of fruits per plant, fruit sweetness (in terms of TSS) was not low as expected. Interestingly, it was significantly higher compared to the intermediate and the wet zones (Table 3). Increase in fruit sweetness under moderate water stress has been reported for tomatoes (Veit-Kohler *et al.*, 1999; Bertin *et al.*, 2000; Nahar and Ullah, 2018), peach (Kobashi *et al.*, 2000), plums (Maatallah *et al.*, 2014) and nectarines (Thakur and Singh, 2012). Sugar levels notably increase under drought stress to affect osmotic potentials and high sugar levels in cell vacuoles helps to produce high turgor pressure (Ma *et al.*, 2017).

Despite excellent physiological performance and yield, fruit antioxidant properties were lowest in the intermediate zone (Table 4), which clearly indicated that plants in this zone were not significantly affected by any abiotic stress conditions. Significantly higher TPC and ACEA in dry and wet zones (Table 4) indicated considerable abiotic stress. Low antioxidant properties of fruits in the intermediate zone on the other hand clearly indicated that the plants at the intermediate zone were not significantly affected by any abiotic stress conditions. Antioxidant compounds in plants increase in response to abiotic stress. Phenolic compounds are powerful antioxidants that protect plants from oxidative stress by scavenging harmful reactive oxygen species (ROS) under different abiotic and biotic stresses (Balasundram *et al.*, 2006; Lattanzio *et al.*, 2006; Li *et al.*, 2012; Kulbat, 2016; Naiko *et al.*, 2019; Samec *et al.*, 2021;). Highest phenol content in the dry zone is undoubtedly due to antioxidant defense activation. An increase in pheno-

lic compounds under drought stress has been also reported for fruits such as mulberry (Khamjad *et al.*, 2021), grapevine (Irani *et al.*, 2021), pomegranate (Farji *et al.*, 2020) and strawberries (Unal and Okatan, 2023). The increase in antioxidant properties may also be in response to high oxidative stress caused by adverse environmental conditions created by nutrient deficiency, high temperature and high UV light (high sunshine hours) (Das and Roychoudhury, 2014). Comparable and high antioxidant properties in the wet and dry zone (Table 4) indicated that plants were under considerable environmental stress. Abiotic stress in the wet zone can be attributed to either excessive precipitation or low phosphorous availability, as discussed earlier. Alfaro *et al*. (2013) reported an increase in polyphenol content and antioxidant activity in murtilla fruits with an increase in rainfall. Extreme precipitation in the area may have also resulted in soil compaction lowering the oxygen levels in the soil leading to activation of the antioxidant defense system. ROS generation also occurs during oxidative stress due to hypoxia (lack of oxygen) which can result from soil compaction (Blokhina *et al.*, 2003; Ali and Alqurainy, 2006). Vergara *et al.* (2012), also found activation of the antioxidant defense system in grapevines in response to hypoxia. P deficiency stress, as discussed earlier, maybe the second reason for noni plant stress and high antioxidant production in the wet zone. Increase in antioxidants in response to phosphorous deficiency has been reported by Tewari *et al.*, (2007); Zhang *et al.*, (2014) and Joel *et al.* (2017). Mineral deficiency stress can also be added to the list of stresses producing high antioxidants in fruits from the dry zone. Tewari *et al.* (2007), showed that antioxidant activity increased in mulberry plants under N, P and K deficiencies.

In conclusion, this study showed that the physiological performance, fruit yield and antioxidant properties of cultivated noni were significantly influenced by water availability and soil nutrient content. The physiological performance including fruit yield was optimum in the intermediate zone which was characterized by a moderate rainfall and fairly good soil properties while poorest physiological performance was in the dry zone. Fruit yield was highest in the intermediate zone with lowest antioxidant properties while it was lowest with highest antioxidant properties in the dry zone. Overall the physiological performance plus fruit yield and antioxidant properties were greatest in the wet zone where plants appear to have a moderate level of abiotic stress. This study also implies that under cultivation, moderate abiotic stress can enhance the antioxidant properties of noni.

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AHS
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Biocontrol of *Fusarium* spp. *in vitro* and in vine cuttings using *Bacillus* sp. F62

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Key words: Antagonism, bioagent, *Fusarium* wilt, vine rootstock.

Abstract: *Fusarium* spp., the causal agent of Fusarium wilt, cause substantial economic losses in viticulture, mainly in tropical regions. This study aimed to assess the biocontrol potential of *Bacillus* sp. F62 against *Fusarium* spp., both *in vitro* and in rootstock cuttings of the SO4 variety. To this end, the *in vitro* antagonism was evaluated through diffusible and volatile compounds synthesized by *Bacillus* sp. F62 on three *Fusarium* spp. isolates. Subsequently, the isolate FusA06-18 was selected for a rootstock cutting experiment. The vine cuttings underwent the following treatments: control, pathogen inoculation (Fus), bacterial inoculation (Bac), and bacterial followed by pathogen inoculation (Bac + Fus). Our findings revealed an average reduction of 39.1% in the mycelial growth of the pathogen through dual culture assay and a decrease of 11.6% in the *Fusarium* spp. radial growth due to the effects of volatile compounds. In the experiment with vine cuttings, applying *Bacillus* sp. F62 reduced the pathogen re-isolation frequency from 81.7% (Fus) to 63.3% (Bac + Fus). Therefore, *Bacillus* sp. F62 effectively suppressed the mycelial growth of *Fusarium* spp. and reduced the Fusarium wilt incidence in vine cuttings of the rootstock 'SO4'.

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1. Introduction

In recent years, the young vine decline and death have affected many vineyards and nurseries worldwide (Gramaje and Armengol, 2011). This syndrome primarily affects vines exposed to stressful conditions, reducing plant productivity and survival in the field (Waite *et al*., 2015; Gramaje *et al*., 2018). Underperforming vines have been found to be affected by trunk and root diseases, disturbing physiological processes such as carbohydrate metabolism, defense responses, and photosynthetic rate (Fontaine *et al*., 2015; Akgül and Ahioğlu, 2019). In this context, *Fusarium* spp. have been associated with the failure or poor establishment of the vineyards, mainly in tropical regions (Halleen *et al*., 2003; Garrido *et al*., 2004; Król, 2006; Ziedan *et al*., 2011; Cruz *et al*., 2014; Abdullah *et al*., 2015; Markakis *et al*., 2017; Ghuffar *et al*., 2018; Reveglia *et al*., 2018; Akgül and Ahioğlu, 2019).

Fusarium spp. are soil-inhabiting pathogens that affect many plant

species, including grapevines (Sotoyama *et al*., 2016). These phytopathogens infect the vines through wounds in the root system, causing root rot. Subsequently, the pathogen promotes xylem obstruction, vascular injuries, and plant wilting due to the interruption of water and nutrients transportation to the shoots (Brum *et al*., 2012; Eljounaidi *et al*., 2016; Markakis *et al*., 2017). Besides, this pathogen can be transmitted through pruning and grafting, infecting the rootstock, graft union, and scion (Akgül and Ahioğlu, 2019). Fusarium wilt mainly affects susceptible vine rootstocks belonging to the *Berlandieri-Riparia* family, including the varieties SO4, Kobber 5BB, and Solferino. Although the 'SO4' rootstock exhibits high adaptability to different soils and climate conditions, ensuring good vineyard yield and fruit quality, it is highly susceptible to Fusarium wilt (Vilvert *et al*., 2016).

Given the difficulties in managing soil-borne pathogens, the limited efficacy, and the environmental risks of synthetic fungicides (Armengol and Gramaje, 2016; Gramaje *et al*., 2018), the use of antagonistic bacteria such as *Bacillus* spp. represent an alternative in the control of Fusarium wilt. These rhizobacteria can colonize plant tissues and vessels, suppressing the proliferation of vascular pathogens (Eljounaidi *et al*., 2016). In addition, rhizobacteria can promote plant growth and enhance crop yield (Legein *et al*., 2020; Morales-Cedeño *et al*., 2021).

In previous research, the rhizobacterium *Bacillus* sp. strain F62 demonstrated the potential to suppress black foot disease by 24.6% in 'SO4' (*Vitis berlandieri* x *V. riparia*) and by 29.5% in '1103P' (*Vitis berlandieri* x *V. rupestris*) rootstock plants obtained through micropropagation. Considering these findings, the present study aimed to evaluate the ability of *Bacillus* sp. F62 suspension to inhibit the mycelial growth of three isolates of *Fusarium* spp. and investigate its biocontrol activity against *Fusarium* sp. isolate FusA06-18 in stem wounds in the susceptible rootstock 'SO4'.

2. Materials and Methods

Microorganism isolates

Three isolates of *Fusarium* spp. (FusA97-11, FusP08-10, and FusA06-18) were isolated from symptomatic grapevines from Brazilian vineyards (Table 1). The rhizobacterium *Bacillus* sp. strain F62 was obtained from the soil in Caxias do Sul, Rio Grande do Sul State, Brazil. All microorganisms were preserved in the collection of the Laboratory of Biological Plant Disease Control at the University of Caxias do Sul, Brazil. Molecular identification of the rhizobacterium was performed by amplifying the *16S rDNA* gene with primers for bacteria domains, according to Sterky and Lundeberg (2000). The sequence exhibited 100% similarity to a pre-existing sequence in the National Center for Biotechnology Information (NCBI) of *Bacillus* sp. F62 with accession number NR 102783.2.

Table 1 - Isolates of *Fusarium* spp. used in the assays

Isolates	Origin (city/country)	Grapevine variety
FusA97-11	Alto Feliz, Brazil	Isabella
FusP08-10	Caxias do Sul, Brazil	Isabella
FusA06-18	Caxias do Sul, Brazil	Yves

Antagonism on mycelial growth of the pathogen

The antagonistic effect of *Bacillus* sp. F62 against *Fusarium* spp. was assessed in two different assays: antagonism through volatile and diffusible compounds. These experiments followed the methodology described by Russi *et al.* (2020). Initially, a single colony-forming unit (cfu) of *Bacillus* sp. F62 was cultured in a flask containing 10 ml of Potato Dextrose (PD) broth. The incubation was conducted on a rotary shaker at 150 rpm and 30 ± 2°C for 12 h. Subsequently, this pre-inoculum was transferred to an Erlenmeyer flask with 100 ml of PD broth and maintained under the same incubation conditions for 24 h. Afterwards, the bacterial suspension was centrifuged (3,500 × g) at 23°C for 5 min. The supernatant was discarded, and the pellet was washed twice with sterile water and resuspended in a 0.85% NaCl solution. The bacterial concentration was adjusted to 1×10^6 cfu ml^{-1} for *in vitro* antagonism and 1×10^8 cfu ml^{-1} for *in vivo* assay. Mycelial discs (5 mm in diameter) of the pathogen isolates were obtained from 10-day-old colonies grown in Potato Dextrose Agar (PDA) medium at 25 ± 2°C, with a 12 h light/12 h dark cycle.

In the antagonism through diffusible compounds, a mycelial disc was placed in a PDA medium plate, and after 24 h, four drops of a bacterial suspension (1×10^6 cfu ml^{-1}) were inoculated around the fungal mycelium. For the antagonism through volatile

compounds, a mycelial disc of the pathogen colony was inoculated in the center of a plate containing PDA medium. In another plate with the same medium, 100 µl of *Bacillus* sp. F62 suspension (1×10^6 cfu ml^{-1}) was uniformly spread. Subsequently, the plates were affixed together and sealed to prevent the loss of the bacterial metabolites. Plates inoculated with the pathogen isolates served as a control. All plates were incubated at 25±2°C with a 12 h light/12 h dark cycle for 14 days. The experiment was performed using a completely randomized design, with ten replicates for each fungal isolate.

Measurements of the colony diameter were performed using a digital caliper, and the data were used to determine the mycelial growth rate (MGR), according to the formula:

$$MGR = \Sigma [(d - dp) / N]$$

where d represents the mean of the colony diameter at the present day, dp represents the mean of the colony diameter from the previous day, and N represents the number of days of plate incubation. The mycelial growth inhibition (MGI) was also determined on the 14^{th} day of the experiment according to

$$MGI = [(dc - dt) / dc] \times 100$$

where dc and dt represent the mean of the colony diameters of control and treated groups, respectively, as described by Oliveira *et al.* (2016).

Biocontrol on rootstock cuttings

Four-year-old dormant cuttings of 'SO4' were obtained from vineyards at Embrapa Grape and Wine, Bento Gonçalves, Rio Grande do Sul State, Brazil. After hydration in distilled water for 24 h, the cuttings (30.0 cm in height) were subjected to hot water treatment at 50°C for 30 min, as described by Lerin *et al.* (2017). Four cuttings were arranged in each plastic pot containing 500 ml of autoclaved substrate (90% sphagnum peat and 10% vermiculite), pH 5.5, amended with 5 g l^{-1} of gradual release fertilizer (5-6 months). The isolate of *Fusarium* sp. FusA06-18 was selected for the *in vivo* assay due to its intermediate behavior in *Bacillus* sp. F62 antagonism.

The experiment was carried out using a completely randomized design, with 60 rootstock cuttings per treatment, according to Haidar *et al*. (2016 a), with modifications. Rootstock cuttings were subjected to surface disinfection with 70% (v/v) ethanol by rubbing with cheesecloth, and then cuttings were wounded with a scalpel above the first basal bud (4 mm in diameter). The trial consisted of applying the following treatments at the wounds: control (40 µl of sterile water), Bac (40 µl of *Bacillus* sp. F62 suspension containing 1×10^8 cfu ml^{-1}), Bac + Fus (40 µl *Bacillus* sp. F62 suspension and a mycelium disc of FusA06-18), and Fus (mycelium disc of FusA06-18). The wounds were covered with plastic film, and the cuttings were maintained in a growth chamber, at 26±2°C, under a 12 h light/12 h dark photoperiod provided by cool white fluorescent tubes. The relative humidity was maintained at 70%. During a 60-day experiment, the cuttings were watered three times a week with sterile water, at 80% of the maximum water holding capacity.

The following morphophysiological responses were assessed after 30 and 60 days: bud number (Budn), leaf number (Leafn), inflorescence number (Infln), and shoot length (Shootl, cm). The pathogen re-isolation frequency (FPR, %) was also determined at the experiment's end. The stems were debarked for pathogen re-isolation, and four fragments were collected 1 cm above and below the inoculation site. These fragments were surface disinfected by sequential immersion in 70% (v/v) ethanol for 30 sec and 3% (v/v) sodium hypochlorite for 1 min. Subsequently, the stem fragments were rinsed three times with sterilized water and then inoculated in plates containing PDA medium. The plates were incubated at 25°C for 10 days. The frequency of *Fusarium* spp. re-isolation was recorded compared to the total number of fragments obtained from each rootstock cutting.

Statistical analysis

The dataset was subjected to Shapiro-Wilk and Levene's tests to assess the normality and homoscedasticity, respectively. In the *in vitro* antagonism and the assay with rootstock cuttings, parametric data underwent one-way ANOVA followed by the Tukey test and non-parametric data were analyzed using the Kruskal-Wallis test followed by the Dunn-Bonferroni test. The frequency of pathogen re-isolation (FPR) between the treatments Fus and Bac + Fus was evaluated using the Mann–Whitney U-test. All analyses were performed with SPSS 22.0 software (SPSS Inc. Chicago, IL), and the threshold for statistical significance was set at $P<0.05$.

3. Results

Bacterial antagonism on mycelial growth

The inhibitory potential of *Bacillus* sp. F62 was determined against three isolates of *Fusarium* spp. (FusA97-11, FusP08-10, and FusA06-18) using diffusible and volatile compounds assays. In the antagonism through diffusible compounds, the bioagent exhibited statistically significant suppression of all isolates of *Fusarium* spp., reducing the mycelial growth rate compared to the control (Table 2, Fig. 1). Among the pathogenic strains evaluated, *Fusarium* sp. isolate FusA97-11 demonstrated the highest mycelial growth rate. The mycelial growth inhibition (MGI), determined on the last day of the assay, ranged from 30.4% (FusA06-18) to 47.1% (FusP08-10).

In the assessment of bacterial antagonism through volatile compounds, there was a statistically significant difference between the treatments (Fus and Bac + Fus). The volatile organic compounds led to a reduction in mycelial growth rate in all the *Fusarium* spp. isolates (Table 2, Fig. 2). The mycelial growth inhibition (MGI) ranged from 8.2% (FusA97-11) to 14.3% (FusP08-10). Although volatile compounds exhibited lower effectiveness in inhibiting the radial growth compared to diffusible compounds, these volatile metabolites not only affected the radial growth of the pathogen but also caused modifications in mycelial morphology (Fig. 3). Regarding the antagonism of *Bacillus* sp. F62 against *Fusarium* spp., both diffusible and volatile compounds promoted a higher suppression against the pathogenic isolate FusP08-10.

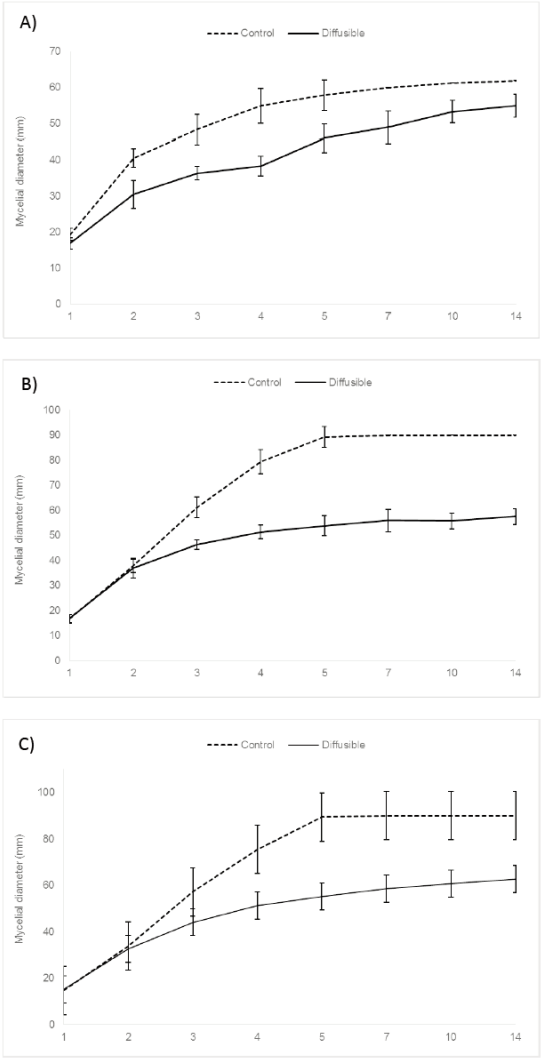

Fig. 1 - Mycelial growth of three *Fusarium* spp. isolates during 14 days of incubation in the antagonism assay through diffusible compounds. A) FusA97-11, B) FusP08-10, and C) FusA08-16

Table 2 - Mycelial growth rate (MGR, mm/day) of three *Fusarium* spp. isolates subjected to the following treatments: *Fusarium* spp. (Fus) and *Bacillus* sp. F62 + *Fusarium* spp. (Bac + Fus), in the antagonism through diffusible and volatile compounds. The mycelial growth inhibition (MGI, %) was determined on the last day of the experiment

Treatments	FusA97-11	FusP08-10	FusA06-18	Mean
		Antagonism through diffusible compounds		
Fus	10.8 ± 0.4 aA	10.4 ± 0.2 aB	10.7 ± 0.2 aAB	10.6 ± 0.3 a
Bac + Fus	6.5 ± 1.3 bA	5.5 ± 0.8 bA	6.1 ± 1.3 bA	6.0 ± 1.1 b
MGI (%)	39.8	47.1	30.4	39.1
		Antagonism through volatile compounds		
Fus	7.3 ± 0.1 aB	7.0 ± 0.2 aC	8.1 ± 0.2 aA	7.5 ± 0.2 a
Bac + Fus	6.7 ± 0.8 bAB	6.0 ± 0.6 bB	7.1 ± 0.5 bA	6.6 ± 0.6 b
MGI (%)	8.2	14.3	12.3	11.6

*Statistical analysis was performed separately in the antagonism through diffusible and volatile compounds.
**Equal lowercase letters indicate no statistically significant difference between the treatments (Fus and Bac + Fus) using t-test (P<0.05). Equal uppercase letters indicate no significant difference among the fungal isolates, using ANOVA followed by Tukey's test (P<0.05).

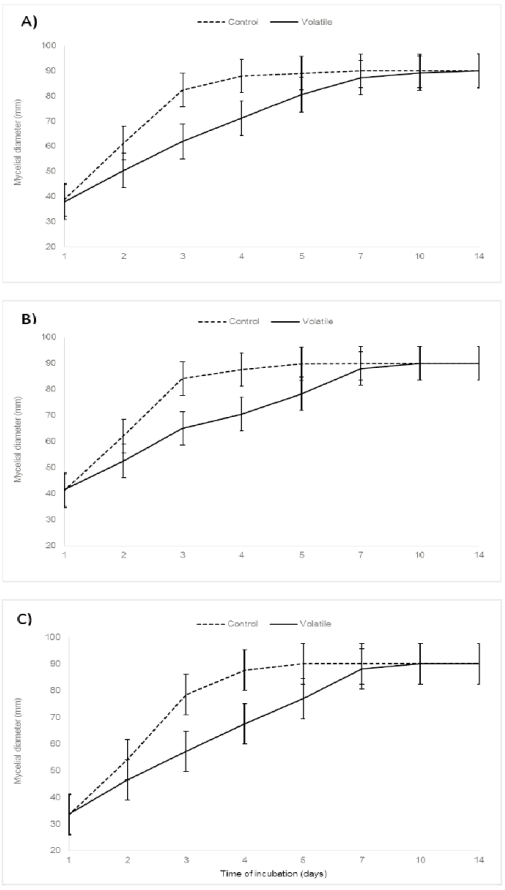

Fig. 2 - Mycelial growth of three *Fusarium* spp. isolates during 14 days of incubation in the antagonism assay through volatile compounds. A) FusA97-11, B) FusP08-10, and C) FusA08-16.

Fig. 3 - Morphology of colonies of *Fusarium* sp. isolate FusA06-18 in the antagonism assay with volatile compounds synthesized by *Bacillus* sp. F62 after 14 days of incubation. The control treatment is on the upper left side of the photograph.

Biocontrol on rootstock cuttings

The bioagent was applied for the biocontrol of *Fusarium* spp. isolate FusA06-18 in stem wounds of 'SO4' cuttings (Table 3). While the inoculation of *Bacillus* sp. F62 in wounds did not improve the growth promotion responses evaluated in rootstock cuttings, it reduced the frequency of the pathogen re-isolation from 81.7% in the Fus treatment to 63.3% in the Bac + Fus treatment (reduction of 22.5% in the Fusarium wilt incidence).

Table 3 - Morphophysiological responses in rootstocks cuttings of 'SO4': bud number (Budn), leaf number (Leafn), inflorescence number (Infln), shoot length (Shootl, cm), and frequency of pathogen re-isolation (FPR, %), subjected to the treatments: control, *Bacillus* sp. F62 (Bac), *Bacillus* sp. F62 + FusA06-18 (Bac + Fus) e FusA06-18 (Fus). The responses were assessed in two different periods: 30 and 60 days post-inoculation

Treatments	30 days post-inoculation				60 days post-inoculation				
	Bud number	Leaf number	Inflorescence number	Shoot length	Bud number	Leaf number	Inflorescence number *	Inflorescence number	FPR **
Control	0.5 ± 0.3	7.3 ± 0.9	2.3 ± 0.6	9.9 ± 4.4	0.6 ± 0.2	9.5 ± 2.3	1.9 ± 0.6	19.8 ± 7.2	-
Bac	0.6 ± 0.2	6.7 ± 1.3	2.2 ± 0.9	9.1 ± 2.5	0.6 ± 0.2	10.5 ± 1.6	1.9 ± 0.4	20.5 ± 4.2	-
Bac + Fus	0.6 ± 0.2	7.1 ± 1.9	2.2 ± 0.6	9.5 ± 2.9	0.6 ± 0.1	10.5 ± 1.3	1.70 ± 0.8	21.7 ± 5.2	63.3 ± 2.1 b
Fus	0.6 ± 0.1	6.9 ± 1.1	2.3 ± 0.9	10.3 ± 3.2	0.7 ± 0.1	10.3 ± 1.7	1.8 ± 0.5	22.7 ± 6.1	81.7 ± 1.5 a

* Different letters indicate statistically significant difference using ANOVA followed by the Tukey's test (P<0.05), except for inflorescence number (Infln) analyzed using the Kruskal-Wallis test followed by the Dunn-Bonferroni test (P<0.05).
** Frequency of pathogen re-isolation (FPR) was subjected to the Mann-Whitney U-test (P<0.05).

4. Discussion and Conclusions

The antagonism activity of the rhizobacterium *Bacillus* sp. F62 against *Fusarium* spp. was evaluated in two experiments, *in vitro* and *in vivo,* with vine cuttings of the rootstock 'SO4'. In the dual culture assay, the bioagent inhibited the growth rate of *Fusarium* spp. through the release of antimicrobial compounds and competition for space and nutrients. This finding is consistent with the observations of Nourozian *et al*. (2006), who reported that two strains of *B. subtilis* inhibited the mycelial growth of *Fusarium graminearum* by 97%. Similarly, Ziedan *et al*. (2010) found that seven strains of *Streptomyces* spp. exhibited notable antagonistic activity *in vitro* against *F. oxysporum*. Santos *et al*. (2016) also observed that a commercial product containing *B. subtilis* (Rizolyptus®) reduced the mycelial growth of six isolates of *Dactylonectria macrodidyma* by approximately 41%.

However, the volatile metabolites produced by *Bacillus* sp. F62 did not suppress fungal growth *in vitro*. This is in line with the findings of Nigris *et al*. (2018), who reported that *B. licheniformis* GL174 did not control the mycelial growth of *Phaeoacremonium aleophilum*, *Botryosphaeria* spp., and *Botrytis cinerea* through volatile compounds, while diffusible compounds inhibited the colonies growth by 60%. Likewise, Gao *et al*. (2018) observed that volatile molecules synthesized by *B. subtilis* CF-3 did not suppress the development of *Macrophoma kuwatsukai* and *Penicillium expansum*, causal agents of apple diseases. In contrast, Rocha and Moura (2013) observed that volatile compounds of *Streptomyces* sp. DFs1315 and *B. subtilis* reduced the colony diameter of *Fusarium oxysporum* f. sp. *lycopersici* by 18.1% and 17.5%, respectively.

Regarding the biocontrol potential of *Bacillus* sp. F62 against *Fusarium* sp., our experiments demonstrated a reduction in the percentage of pathogen re-isolation. Likewise, Haidar *et al*. (2016 a) reported that eight bacterial strains isolated from French vineyards effectively controlled *P. chlamydospora*, reducing the frequency of pathogen re-isolation from 31.4 to 38.7% compared to the control. Additionally, several bacterial strains, especially *Pantoea agglomerans*, significantly reduced the length of necrosis caused by *N. parvum* by 32.3% and 43.5% on grapevine cuttings (Haidar *et al*., 2016 b). Wicaksono *et al*. (2017) also observed that two isolates of *Pseudomonas* sp. inoculated onto wounds in grapevine cuttings cv. Sauvignon Blanc inhibited two botryosphaeriaceous species, *Neofusicoccum luteum* and *N. parvum*, and reduced lesion length caused by 32-52% compared to the untreated control.

Numerous studies have reported the ability of rhizobacteria to improve plant growth through nutrient solubilization, production of siderophores and phytohormones, such as auxins, gibberellins, and cytokinins (Olanrewaju *et al*., 2017). Rolli *et al*. (2017) tested the potential of fifteen rhizobacteria obtained from grapevines, olive trees, and pepper plants to enhance the growth of 'Syrah' grafted on '1103P' rootstock and 'Cabernet Sauvignon' grafted on 'SO4' rootstock in the field. The results demonstrated rapid colonization of the rhizoplane and root system of grapevine by the rhizobacteria. Moreover, bacterized plants showed longer shoots, larger diameters, and higher number of nodes on shoots.

In the current study, the application of *Bacillus* sp. F62 in artificially induced injuries did not increase plant growth of 'SO4' cuttings. Nevertheless, the inoculation of this same bacterium by soil drenching in cuttings of 'SO4' improved plant development by increasing the length of the primary shoot, the number of nodes in the primary shoot, and the total number of nodes (Russi *et al*., 2020). Wicaksono *et al*. (2017) evaluated the efficacy of two methods for bioagent inoculation: stem wounding and soil drenching. The authors found that *Pseudomonas* sp. colonized internal tissues of 'Sauvignon Blanc' cuttings when inoculated by wounding, but the bacterial proliferation failed when soil inoculated. As a result, plant morphological barriers and released toxins can prevent tissue colonization by some bacterial strains, reducing their effect in the phyllosphere (Balmer *et al*., 2012). According to Compant *et al*. (2010), tissue colonization is influenced by several factors, such as the pattern of plant exudates, nutrient availability, rhizobacteria growth rate, bacterial-host interactions, stress conditions, and plant genotype, which may explain the differences observed among these studies. Furthermore, the plant tissue inoculated and the phytopathogen strain can influence the antagonistic potential of rhizobacteria (Haidar *et al*., 2016 b).

In summary, this study demonstrated the effectiveness of *Bacillus* sp. strain F62 in controlling three isolates of *Fusarium* spp., inhibiting mycelial growth through volatile and diffusible compounds. Moreover, the rhizobacterium reduced the incidence of Fusarium wilt in 'SO4' vine cuttings that were

artificially infected with the pathogen. Consequently, *Bacillus* sp. F62 holds promising potential as a biocontrol agent for suppressing *Fusarium* spp. in susceptible vines.

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Review paper

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Advances in Horticultural Science

Inhibition of bleaching of stored red hot pepper through appropriate postharvest technologies and practices

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Key words: Capsicum *spp*., carotenoids, color fading.

Abstract: The colour qualities of hot red pepper are the major issue in pepper value chain. Due to poorly coordinated scientific evidence, information on the factors causing colour loss and the inhibition mechanism is not well known. Therefore, this review paper aimed to summarize the inhibition mechanism of stored red hot pepper bleaching through appropriate postharvest technologies and practices. The information in this paper was gathered from a variety of sources, including journal articles, books, book chapters, workshop proceedings, FAO reports, and AOAC official methods of analysis. According to these studies, carotenoids, surface colour, and extractable colour (ASTA value) are the primary colourants that define hot red pepper. The findings demonstrate that low-temperature drying methods, such as open sun drying, are best for preserving the red hot pepper powder's colour quality, while higher temperatures cause the colour to darken. Blanching, the use of desiccants ($CaCl_2$), and chemical dipping are pretreatments that preserve the best colour quality by hastening the drying time. Similarly, storage of red hot pepper powder at lower temperatures (5°C) resulted in less colour degradation. In other words, materials used for packaging that have a high barrier to light, moisture, and air, such as laminated aluminium, amber or black polyethylene, and high-density polyethylene, maintained a higher level of colour quality. Through their influence on drying and processing times, breeding technologies, varieties, and maturity level also impact colour quality. In conclusion, the colour quality of red hot pepper is highly influenced by environmental, biological, and processing methods. It is, therefore, critical to use appropriate drying and pretreatment techniques, storage time, well-managed storage temperature, appropriate processing methods and packing materials, and improved agronomic practices for the sustainable management of colour fading and adulteration that can occur throughout the value chain.

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1. Introduction

Pepper (*Capsicum*spp.) is one of the oldest and most widely used crops. Evidence from prehistoric times show that *Capsicum* species were used as a spice as early as 5500 BC. Central and South America are the origins of the *Capsicum* spp., with Peru and Mexico believed to be the

second centres of origin, subsequently it spread into the New World Tropics before being introduced into Asia and Africa in 1493 (Bosland and Votava, 2000). It was introduced to Europe by Christopher Columbus in the 15th century, and it later spread to Africa and Asia via the flourishing trade routes of Spain and Portugal at the time. As it spread around the world, it was quickly adopted and used as a spice, giving rise to many regional varieties (Jarret *et al.*, 2019). Today, hot pepper (*Capsicum annuum* L.) is the world's most important vegetable cultivated after tomato, and it is used as a vegetable, spice, or condiment in fresh, dried, or processed products (Acquaah, 2009). Asia accounts for approximately 65% of global pepper production, whereas the United States, Europe, and Africa each contribute 13.3%, 11.9%, and 10.1%, respectively (FAO, 2019).

Red peppers have been used as food additives for several years. They are used fresh as green, red, and multi-colored whole fruits in sauce, paste, canning, and pickling; dried spice (whole fruits and powder); and as an ornamental (Batiha *et al.*, 2020). It is also used as a medicinal plant to treat and relieve pain due to its antioxidant, immune-modulating, antibacterial, and anticancer properties (Maji and Banerji, 2016). It also prevents gastrointestinal problems such as flatulence, loss of appetite, gastroesophageal reflux disease, and gastric ulcers (Kim *et al.*, 2014). It also reduces oxidative stress, inflammation, and body weight, and is used to treat dyspepsia, as well as antiatherosclerotic, antidiabetic, and antihypertensive medications (Baenas *et al.*, 2019).

Red pepper carotenoids are also beneficial to humans in a variety of ways, including as sources of natural food color and provitamin A (Villa-Rivera and Ochoa-Alejo, 2020). Moreover, ground pepper and oleoresins are used to improve the color and flavour of soups, stews, sausage, cheese, snacks, salad dressing, sauces, pizza, confectionaries, and beverages (Arimboor *et al.*, 2015). The oleoresin, which is used to replace synthetic food colorants extracted from red carotenoids, is now a source of income for pepper-producing countries (Melgar-Lalanne *et al.*, 2017). It also contains vitamin C, phenolic compounds, proteins, fat, carbohydrate, dietary fibre, sodium, potassium, calcium, magnesium, iron, zinc, copper, and manganese (Dobón-Suárez *et al.*, 2021).

Natural food colourants are preferable to synthetic food colourants because they reduce the risk of synthetic food colourants to human health (Arimboor *et al.*, 2015). However, fading of the colour in red pepper is becoming a major issue due to a variety of complex reasons; as a result, fraudulent traders are adulterating the red pepper products with other artificial products such as Sudan dyes, water and oil soluble dyes, oils, and Rhodamine (Osman *et al.*, 2019). The colour fad is also caused by improper post-harvest handling and pepper production technologies (Hyderabad-Avanti *et al.*, 2019). It is also influenced by a lack of sufficient agricultural inputs, postharvest technological equipment, and knowledge, heat generated during grinding, drying techniques, red pepper powder particle size, water activity and interaction with moisture, type of packaging materials, storage periods, maturity stage, relative humidity, and light (Kasampalis *et al.*, 2022). Several novel drying technologies and postharvest handling systems were developed to preserve the nutritional and colour qualities of hot red pepper products (Getahun *et al.*, 2021). Perhaps the information on the bleaching of stored red hot pepper, the factors that cause the bleaching, and the inhibition mechanisms are not well summarized for protracted management of the problem and ensuring the nutritional importance of the red hot pepper. This paperis, therefore, aimed to review the appropriate postharvest technologies and practices that inhibit the bleaching of stored red hot pepper.

2. Materials and Methods

Literature was gathered between June 2022-September 2022. This review relied on journal articles, books, book chapters, workshop proceedings, FAO reports, AOAC official methods of analysis, bulletins, legal papers, and unpublished reports, including M.Sc. and Ph.D. dissertations. We used the NICE guidelines: the manual (NICE, 2014), Systematic Reviews: CRD's guidance for undertaking reviews in health care (CRD, 2009), The Cochrane Handbook (Lefebvre *et al.,* 2011), and The Joanna Briggs Institute Reviewers' Manual (Santos *et al*., 2018) guidelines to conduct our literature search. In total, 1406 pertinent sources of information were found after a thorough investigation of databases and websites. The databases used in this study were Food Science Technology Abstracts (FSTA), BIOSIS Citation Index, PubMed (Medline), Web of Science, CAB Abstracts, Cochrane Library, Science Direct, Wiley Online Library, Scopus, and Google Scholar. Additionally, theses and dissertations were gathered

from institution websites. The related electronic literature was obtained using a Boolean search technique. Based on their direct relevance to the review's title, 107 articles from the entire corpus of downloaded literatures were used (Cooper *et al*., 2018).

3. Major red pepper colors and measurements methods

Carotenoids

Carotenoids are yellow-orange-red lipophilic pigments found in photosynthetic plants, algae, and microorganisms (Mezzomo and Ferreira, 2016). It is a fat-soluble pigment found in animals and plants that contains over 700 compounds that exhibit red, orange, and yellow colours (Jaswir *et al.*, 2011). It is a colour basal structure derived from tetraterpenephytane (C40), and any changes to this backbone result in various types of carotenoids (Mezzomo and Ferreira, 2016). Carotenoids such as β -carotene, zeaxanthin, and lutein are primary carotenoids that are directly involved in photosynthesis, whereas lycopene, α-carotene, and capsanthin are secondary carotenoids that play no role in photosynthesis (Arimboor *et al.*, 2015). Overall, it is a good natural source of colour and is commercially used as food colourants and feed additives (Jaswir *et al.*, 2011).

Carotenoids such as capsorubin, cryptoxanthin, and zeaxanthin are found in peppers as fatty acid esters. The carotenoids formed in the fruit during ripening are primarily responsible for the colour of red pepper. Red pepper carotenoids have over 50 different structures (Arimboor *et al.*, 2015). Capsanthin, capsorubin, and their isomers are the most important pigments, accounting for 30-60% and 6-18% of the total number of carotenoids in peppers, respectively (Nadeem *et al.*, 2011). It is also reported that capsanthin contributes 45.27% of total carotenoid, while antherazanthin and capsorubin contribute 8.95% and 11.45%, respectively (Ko *et al.*, 2022). The type of carotenoids depends on environmental conditions, ripening stage, cultivar and agro-climatic conditions, and so on (Kim *et al.*, 2021). Processing methods such as drying, seed removal, and grinding are also cited as major contributors to the carotenoid content of fruits (Loizzo *et al.*, 2013).

The wide structural diversity, as well as possible isomeric forms and derivatives, have been used to analyse carotenoid content, but the analysis method is difficult (Yan *et al.*, 2020). Sample preparation, extraction with various solvents, purification, saponification, separation, detection, and quantification are all common analytical steps. However, the instability associated with carotenoids' characteristic conjugated double bond structure necessitates the incorporation of control measures such as minimizing the possibility of carotenoid loss during analysis (Borba *et al.*, 2019). The precautions include performing laboratory operations in dimmed, yellow, or red light and performing sample preparation, extraction, evaporation, and saponification steps in the presence of antioxidants under a protective nitrogen or argon environment at a temperature below 40°C, as well as storing the samples/extracts in an inert atmosphere at temperatures around -20°C (Arimboor *et al.*, 2015).

Surface color

Surface colour measurements are used to specify colours perceived by the human eye. Different surface colour measurement techniques have been developed to lessen the challenge of an object's colour being perceived differently by various observers. This is due to the fact that various factors, including the observer's sensitivity, the size of the object, the light source and illumination, the background colour and contrast, and the angle at which the object is viewed, affect how each observer comprehends the colour they observed (Yang *et al*., 2018). Since the late 1920s and 1930s, the relative eye's sensitivity to light was recognized as a standard observer trait (MacDougall, 2010). The CIELAB (International Commission on Illumination's Lab) colour system is used to ascertain the surface colour of red peppers (Pathare *et al*., 2013). Hunter colour parameters have also been used to determine the surface colour of red pepper (Sharangi *et al*., 2022). The Hunter Lab System has a more uniform colour space than CIE. Furthermore, the surface colour of the hot red pepper is determined by the variety, maturity stage, pretreatments made during processing and drying techniques used (Sharma *et al*., 2015).

Extractable color

Extractable colour is a spectrophotometer-measured total pigment content expressed in ASTA units. The current procedures for measuring the extractable colour in dehydrated capsicums and oleoresins were developed by the Association of Official Analytical Chemists. Higher ASTA colour units indicate a brighter red colour and product acceptability (Babu *et al.*, 2014). However, because the extraction pro-

cess takes 16 hours, the ASTA method is completely objective, destructive, and time consuming (Monago-Maraña *et al.*, 2022).The extractable colour of the pepper is heavily influenced by storage conditionsand temperature (Belović *et al*., 2014), pretreatment method and maturity stage (Bhandari *et al*., 2013).

4. Kinetics of red hot pepper color fading and determinant factors

Red pepper colour degradation is caused by oxidation caused by singlet oxygen and other reactive species such as O_2, H_2O_2 and OH (Ding *et al.*, 2015). The oxidation process results in the complete loss of carotenoids. It is also caused by carotenoid isomerization from *trans* to *cis* form, which is accelerated by exposure to relatively higher heat, acids, and light (Provesi and Amante, 2015). Unlike oxidation, isomerization results in colour saturation rather than a complete loss of carotenoids (Song *et al.*, 2017). The following sections go over some of the factors that contribute to red pepper colour fading.

Light

Light has a significant impact on the colour quality of peppers both during cultivation and storage. Crop exposure to direct sunlight in the cultivation field has a negative impact on the colour and final product of the pepper. For example, a pepper grown and kept in a greenhouse for less than 200 days revealed a high extractable colour (Gómez *et al.*, 1998). Similarly, prolonged exposure to sunlight resulted in a decrease in C* values and an increase in h ab values, indicating a decrease in vividness (saturation) and an increase in yellowness in tandem with a decrease in redness, because changes in C* values are directly related to colour stability (Pathare *et al.*, 2013). The carotenoid contents in fruits grown in shaded greenhouses were also significantly higher than those grown in unshaded greenhouses and fields (Keyhaninejad *et al.*, 2012). Open sun drying with long processing times was also detrimental, resulting in losses of 79% capsaicinoids and 24.6% capsaicin (Topuz *et al.*, 2011). In comparison to frozen and hot air-dried peppers, chilli peppers dried in the open sun also preserved less bright red colour and ascorbic acid (Toontom *et al.*, 2012). Similarly, 50% ASTA colour values were reduced in Korean red pepper powders exposed to sunlight for 42 days (Kim *et al.*, 2015). In general, higher oxygen exposure and intense vaporisation from the pepper's surface cause pigment decomposition during open sun drying (Sharangi *et al.*, 2022).

Water activity

"Water activity" is a thermodynamic measure of water in material that is calculated by dividing the vapour pressure of the water in a sample by the vapour pressure of pure water at a given temperature, which ranges from 0.1-1 (Lewicki *et al.,* 2004).It gauges how effectively the water in the reaction can participate in a chemical or physical process. Moreover, it has a negative impact on the colour and safety of red pepper (Rhim and Hong, 2011). It has been reported that 0.4-0.6 water activity values result in less colour loss (Lee, 2012), but higher water activity develops brown and tarnish-black colour, indicating that the degradation of carotenoid pigments, non-enzymatic browning index, ASTA values, and surface colour (Rhim and Hong, 2011). In other words, water activity between 0.4 and 0.6 reduces red pepper surface colour deterioration (Lee, 2012). Moreover, high water activity at high storage temperatures exacerbates the level of colour fading. Therefore, storing red pepper powder below 25°C, below medium ranges of water activity, and between 10-14% moisture content to maintain red pepper colour quality (Rhim and Hong, 2011). For instance, storing red peppers powder at water activity below 0.3, in a nitrogen atmosphere and/or reducing the package free space volume and lowering storage temperature improves the carotenoid content (Lee *et al.*, 1992). An increasing trend of colour stability was also observed with increasing water activity values during pepper powder storage, with the greatest stability observed at a water activity of 0.64 (relative moisture, 14%), but increasing the moisture level above this value caused a significant reduction in colour intensity (Kanner *et al.*, 1977).

Drying and storage temperature

Temperature is an important factor that contributes to the degradation of red pepper colour quality by causing oxidation of carotenoid pigments due to heat destruction and lipid oxidation at high temperatures (Maurya *et al.*, 2018). It also results in the loss of volatile compounds and nutrients like vitamins C, capsaicinoids, phenolic compounds, antioxidant capacities, and other physicochemical properties. For example, colour reduction of pepper powder

is observed to be rapid at high temperatures even when moisture content is low during storage (Kim *et al.*, 2004). Temperature and relative humidity above ambient storage conditions also degrade the colour of pepper, but pepper powder stored in refrigeration and freeze showed a minimal extractable and surface colour loss (Addala *et al.*, 2015).

In other words, the drying kinetics of red and yellow chilli peppers showed a decreasing trend of red colour decomposition at lower drying air temperatures (less than 55°C) (Andrade *et al.*, 2019).For instance, yellow sweet peppers dried in a microwave convective dryer showed a 3.5-fold increase in red colour decomposition with increasing microwave power and temperature due to isomerization of carotenoids at high temperatures (Swain *et al.*, 2014). However, carotenoids are more stable at high temperatures in pungent cultivars because capsaicinoids in pungent varieties affect the chemical interactions of carotenoids in red pepper tissues, providing significant protection against thermal destruction. Thermal destruction and lipid oxidation, on the other hand, can be inhibited by endogenous antioxidants such as phenols, vitamin C, and E. For example, heat has reduced the carotenoid degradation of dried pepper prior to milling to greater than 2 mg/g, but pungency cannot be guaranteed for more than 3 storage months (Daood *et al.*, 2006).

Processing methods

The red pigment of red pepper is also affected by processing methods and conditions. Furthermore, the kinetics of colour degradation during processing and storage are temperature and water-activity dependent. For example, the colour of ground pepper deteriorated faster than that of non-ground pepper during storage, indicating that powdering reduces carotenoid concentration (Carnevale *et al.*, 1980). Exoquin and irradiation-treated samples also retained more red colour of the pepper powder after six months of storage (Addala *et al.*, 2015). Similarly, after 12 months of storage, red pepper with 10% seed had higher colour stability than controls (Van Blaricom and Allen, 1953). Dry-heat cooking methods such as stir-frying and roasting are also preferred over moist heat cooking methods such as boiling and steaming to retain the nutrient compositions and antioxidant properties of red pepper (Wang *et al*., 2017).

Pepper seed addition

The initial carotenoid concentration of extractable colour is also strongly influenced by the extent of powdering and seed inclusion. The inclusion of seeds reduces the carotenoid content because seeds have little carotenoid content and are highly affected by water activity, packaging atmosphere, storage temperature, and pepper treatment (Lee *et al.*, 1992). In other words, adding seed to the flesh slows the rate of colour loss, but because the seed dilutes the initial colour, it reduces the overall shelf life (Klieber and Bagnato, 1999). The addition of 10% seed to sesame-treated samples also improved red pepper colour stability even after a 12-month storage period (Van Blaricom and Allen, 1953).

Variety

The studies revealed that cultivar and growing conditions all have an effect on the degree of pepper coloration. For example, a significant difference in total carotenoid content was observed among different pepper varieties grown in Nigeria, with genotypes UNS3 and Nskyre exhibiting the highest total carotenoids content and genotype Tatase containing high-carotene content (17.30±0.35 mg/100 g) (Abu *et al.*, 2020). Color value difference was also observed among three red hot pepper varieties grown in Ethiopia, with Marakofana having the highest ICU value (648331±31673)(Kinfe, 2009). The colour of hot red pepper pods also varied significantly among Dilla-grown varieties, with Melkazala having a darker-red pod colour than Marekofana (Teferi *et al.*, 2015). Similarly, ASTA values ranging from 2-296 were reported for local and exotic hot pepper genotypes grown in the same conditions in Ethiopia, indicating that variety influences hot red pepper colour (Aklilu *et al.*, 2018).

Fruit ripening at harvest

Pepper colour varies greatly depending on maturity stage due to its carotenoids content; unripe fruit can be green, yellow, or white, turning to red, dark red, brown, and sometimes black when mature (Mohd-Hassan *et al.*, 2019). Capsanthin, capsorubin, and cryptocapsin are keto carotenoids that produce a brilliant red colour, whereas -carotene, zeaxanthin, violaxanthin, and -cryptoxanthin produce a yellow-orange colour (Arimboor *et al.*, 2015). In other words, red peppers contain β-cryptoxanthin, capsanthin, capsorubin, β-carotene, antheraxanthin, violaxanthin lutein and α-carotene, while yellow colored peppers contains β-carotene, zeaxanthin, antheraxanthin, violaxanthin lutein and α-carotene, but

brown colored peppers have green color (chlorophll *b*) and lutein (Mohd-Hassan *et al.*, 2019).

Ripe pepper fruits of various varietiesexhibit a wide range of colours ranging from white to deep red (Mohd-Hassan *et al.*, 2019). For example, total carotenoids content increased with maturity stage, with red sweet pepper having the highest total carotenoids compared to orange, yellow, green, and white cultivars (Gnayfeed *et al.*, 2001). Total carotenoids also increased up to 24 fold with increasing ripening, with deep red pepper containing the most total carotenoids, followed by faint red and colour breaks (Markus *et al.*, 1999).

The apparent colour values of Marekofana varieties grown in Ethiopia also varied according to maturity stage, with green pepper having the highest L* (38) and b (23.3) values (Getahun *et al.*, 2020). Similarly, the after-ripened spice paprika had the highest ASTA colour value when compared to the early and late harvest stages (Sipos *et al.*, 2010). The pepper powder produced from fruits left to dry on the plant (40-25% moisture content) had also the highest ASTA colour value (270 ASTA unit), but decreased to 160 ASTA unit in succulent fruits immediately after red-ripening (Kanner *et al.*, 1977). However, the green matured stage of hot red pepper (Bhandari *et al.*, 2013) cultivars had higher b-carotene content than the ripe red stage.

5. Inhibiting red hot pepper bleaching via postharvest technologies and practices

Drying method

Drying red pepper before processing is a common practice around the world to maintain colour quality (Yang *et al.*, 2018). Open sun drying is the most widely used drying technique in developing countries and produces the best red pepper colour when compared to hot air and infrared drying, but infrared drying significantly improved drying time at the same drying temperature (Cao *et al.*, 2016). For example, sun-dried paparika contained more carotenoids than paparika dried in a hot air oven, refractive window, or freeze drier (Topuz *et al.*, 2011). The convectional drying method, on the other hand, resulted in colour darkening and a decrease in vitamins and bioactive compounds (Guiné, 2018). In other words, as compared to other unconventional dryers, solar drying is generally more cost-effective, and consistent, and does not rely on weather conditions (Tiwari, 2016). In contrast to sun drying, reflection window drying is less expensive, takes less time to dry, and improves product quality (El-Hamzy and Ashour, 2016).

Hot air drying is also preferred over sun drying due to its rapid, massive, uniform, and sanitary drying; however, due to its higher temperature requirement, nutrient degradation and a lower rehydration ratio have been reported (Guo *et al.*, 2021). Similarly, the highest colour retention was found in red pepper dried at 65°C in oven-dried red pepper at the shortest drying time (480 min), but prolonged sun-dried products had the hardest texture (Sharangi *et al.*, 2022). Dry heat processing is also believed to preserve the colour of red pepper better than wet processing (Rybak *et al.*, 2020).

Drying pretreatments

Pretreatments are used to reduce drying time, increase energy efficiency, and improve product quality (Srimagal *et al.*, 2017). Several pretreatment methods were used to reduce drying time while retaining the red colour and other nutritional properties of red pepper. The most commonly used pretreatment techniques in drying red pepper are ethyl oleate and microwave blanching (Srimagal *et al.*, 2017), hot water blanching, the use of desiccants ($CaCl_2$) (Romauli *et al.*, 2021), and chemical dip (Guiné, 2018). Of these, steam blanching produced more colour and other bioactive compounds than hot water blanching (Rybak *et al.*, 2020). Similarly, ohmic heat treated pepper and bell pepper blanching in boiling solution containing 1% sodium hydroxide and 0.25% magnesium carbonate for 3 minutes and drying at 50°C air temperature (Singh *et al.*, 2000) produced better colour quality than the other methods. The highest red pigment and drying kinetics were also observed in OH-pretreated pepper powder as compared to hot Water Blanching because OH pretreatment and drying at 70°C reduce drying time while retaining red pepper quality (Incedayi, 2020).

The use of 2% ethyl oleate and 5% K_2CO_3 solution dip pretreatments at 50°C during hot air dying of red pepper dried also exhibited the highest Hunter L (lightness), *a (redness), and *b (yellowness) values than untreated peppers (Máximo *et al.*, 2017). Similarly, microwave blanching followed by brine solution dipping improved the drying rate and retained more beta carotene content in the samples (Delfiya *et al.*, 2018). The ASTA and surface colour values of the rehydrated peppers were also greatly improved by pretreatment with a solution containing

calcium, salts (10%), and sodium metabisulfite prior to hot-air, oven (50-80°C), or microwave drying at 60-180 W (Vega-Gálvez *et al.*, 2008). The surface colour of the pepper powder made from sliced chilli pods without the pedicle and pretreated with 0.01% KMS (Sarker *et al.*, 2012) and bell pepper shreds dried in a solar polytunnel drier after pretreatment with a KMS 0.20%+CA 0.50% solution (Sharma *et al.*, 2015) was also better than the control. Ethoxyquin-treated and irradiated samples also retained a better extractable colour after six months of storage (Addala *et al.*, 2015). Similarly, red pepper powder blanching in the microwave, infrared, and high-humidity hot air impingement had the highest red pigments when compared to hot water treatment (Wang *et al.*, 2017) (Table 1).

Storage time and temperature management

Lower temperatures are recommended to prevent red pepper colour loss during storage (Rhim and Hong, 2011). For example, red pepper powders had the same ASTA colour value, capsanthin content, and redness (a*) when stored at -5 and -20°C than 20°C, 2°C, and -1°C storage temperatures (Choi

Table 1 - Effect of pretreatment on the colour quality of red hot pepper

Pretreatments	Drying method	Recommendation	References
Sample without pedicle, cut longitudinally and treated with 0.01% KMS; sample without pedicle and sliced; sample without pedicle as a whole; sample with pedicle as a whole	Sundrying	The powder without pedicle, sliced into two parts along the length and treated with 0.01% KMS maintained better sensory quality	Sarker *et al.* (2012)
Blanching at 93°C in plain or in 2% NaCl solution for 4 min	Solar dryingand in the open sun	Blanching followed by drying in natural convection solar dryer scored better color acceptability	Owusu-Kwarteng *et al*. (2017)
Submerging for 10 min at 25°C in an aqueous solution of 15% (w/w) NaCl, 1.0% (w/w) CaCl2 and 0.3% (w/w) Na2S2O5; blanching in hot water at 85 °C	Refractancewindow™ drying; oven drying; sun drying	Blanching prior to refractance window drying enhanced stability of pepper colorfor 12 months storage period	El-Hamzy and Ashour (2016)
Dipping in chemical solutions (w/v):2% ethyl oleate;2% ethyl oleate+2% NaOH; 2% ethyl oleate+2% NaOH+4% potassium carbonate. Dipping temperature; 23°C; 60°C	Greenhouse drying; open sun drying	Dipping red peppers in 2% ethyle-oleate + 2% NaOH + 4%K_2CO_3 solution at 60°C resulted in best color retention	Ergüneş and Tarhan (2006)
Blanching with hot water; blanching with steam at 98◦C for 3 minutes; Ultrasonic Treatment (US); Pulsed Electric Field Treatment (PEF); combined methods	Vacuum drying (10 mPa, 70°C for 24 h)	Better retention of vitamin C and carotenoids content was observed for the treatment combination based on sonication with a pulsed electric field	Rybak *et al.* (2020)
Control (without any pre-treatment); blanching in boiling water for 3 min; blanching + soaking for 5 min in the following chemical solutions: KMS (potassium meta bisulphite) 0.25%; KMS 0.35%; CA (citric acid) 0.3%; CA 0.6%; KMS 0.2% + CA 0.5%; KMS 0.3% + CA 0.25%	Dehydrator a 58±2°C till constant weight obtained	Blanching of bell pepper shreds in boiled water followed by pre-treatments with KMS 0.20% + CA 0.50% at 55-60°C resulted higher colour stability	Sharma *et al*. (2015)

et al., 2018). Furthermore, as the storage period increased up to 12 months at high temperature, the overall freshness, redness, hot flavour, moisture release, and edibility decreased (Wang *et al.*, 2017).

Chilli powders stored at 5°C retained vitamin C and colour for up to 6 months without the use of any synthetic preservatives. Additionally, chilli pepper powders packed in flexible foil and stored at 5°C retained more vitamin C and colour without the addition of preservatives for up to 6 months as compared to high-density polypropylene storage (Al-Sebaeai, 2017). In other words, red pepper lightness (CIE L*) and yellow colour intensity (CIE b* value) increased, but red colour (CIE a* value) decreased, and the hue angle shifted from red orange to orange-yellow after three years of storage at 21.5°C (Belović *et al.*, 2014). For samples stored at 35°C for 42 days, the L*, a*, b*, delta E, visual colour acceptability, and total carotenoids also revealed a decreasing trend in Candida red, Candida orange, and Candida yellow (Kim *et al.*, 2015).

Similarly, minimum (5%) extractable colour and Hunter L, a, b values change observed for red pepper stored under refrigeration and freeze conditions for both pretreated and non-treated samples at 6 months storage time compared to the room (22°C) and elevated temperatures (35°C and 80%) (Addala *et al.*, 2015). Total carotenoid content loss is also facilitated by storing the microencapsulated NAEC at temperatures above 35°C (Guadarrama-Lezama *et al.*, 2014). In general, the longer the storage period, the lower the storage temperature required, and vice versa (Choi *et al.*, 2018).Red pepper can also be stored at low temperatures (0-5°C) for up to 6 months and frozen (-5°C) for longer than 6 months (Wang *et al.*, 2017).

Packaging materials

Packaging is a method of providing proper environmental conditions for food during storage, and the materials used in packaging are determined by the nature of the product, storage, and handling conditions (temperature, humidity, risk of physical deterioration) (Marsh and Bugusu, 2007). The air composition inside the package is determined by the nature of the packaging materials, which affects the rate and extent of nutrient loss and microbial activity (Amit *et al.*, 2017). Natural jute sacks, for example, were the most widely used bags to pack pepper pods in the world, with significant retention of product quality, but red peppers stored in jute bags were vulnerable to aflatox in contamination as compared to pods packed in polyethylene bags (Iqbal *et al.*, 2015). Hot peppers stored in LDPE film also spoiled faster than unpacked control fruits at 18-20°C and 28-30°C storage temperatures due to excess water vapour accumulated inside the package (Mahajan *et al.*, 2016). Similarly, 77.4% of the initial amounts of health and nutrition-promoting compounds in pepper pods stored in natural jute at room temperature for five months were preserved, but capsaicinoids and antioxidant levels gradually decreased in dry pepper pods over the long storage period (Iqbal *et al.*, 2015).

Purdue Improved Crop Storage (PICS) bags have recently been introduced to store agricultural products because they provide a better barrier to light, air, and moisture. Other storage materials used to pack dried peppers include High-density Polyethylene (HDPE) bags, which have a high moisture barrier and thus cause less deterioration to dried products than natural jute sacks (Sachidananda *et al.*, 2013). Five months of storage in synthetic HDPE plastic bags at room temperature preserved 87.3% of the initial amounts of total carotenoids and ascorbic acid (Iqbal *et al.*, 2015).

Similarly, due to the high rate of migration of water vapour from the storage environment into these packaging materials, aluminium foil, and High-Density Poly Ethylene bag had no effect on water activity for the entire storage period for both red and yellow capsicum pod up to 60 days as compared to polypropylene and Poly Ethylene bags (Sachidananda *et al.*, 2013). The studies also revealed that red pepper pods stored in laminated aluminium for more than 120 days showed the least difference in total carotenoid content and browning index, followed by high-density polyethylene (HDPE) and polypropylene (Weil *et al.*, 2017). This is due to the low light transitivity through the laminated aluminium foil, which results in less reduction of the photo-degradable carotenoid pigments. In general, aluminium foil laminate was generally recommended for storing chilli pepper powder because it is a barrier to light, moisture, and air. As alternatives, amber or black polyethylene, high-density polyethylene, and Saran/Cello/Saran poly laminate pouches are recommended (Sachidananda *et al.*, 2013). Table 2 also illustrates some packaging-related effects on the colour quality of red hot peppers.

Table 2 - Effect of packaging materials on the colour quality of red hot pepper

Packaging materials	Type	Conservation method	Recommendation	References
Polypropylene; aluminum laminated pouch; woven polypropylene bags	Red chilli powder	With and without vacuum pumping storage at 5±0.5°C and 26±1°C for 12 months	Less color loss was recorded at 5±0.5°C and in laminated bags under vacuum at both storage temperature	Prerna *et al*. (2019)
Aluminum; LDPE; HDPE; paper bags; polythene line gunny bags; gunny bags	Dried red pepperpod	Storage for two months under ambient conditions	Laminated aluminium film retained highest color, ascorbic acid and oleoresin	Anjaneyulu and Sharangi (2022)
Polypropylene packages with thickness of 3, 4, 5, 12.5, 20 and 30 micron	Ground chilli pepper	Storage at room temperature (28±2°C) for two months	Polypropylene films with thicknesses of 30 and 20 microns exhibited enhanced preservation of carotenoid content	Akusu and Emelike (2019)
Low density polythene pouch; PET jar; laminated film pouch	Green chilli pepper slice	Storage for 28 days after 7% K2S2O5 pretreatment + blanching for 1.5 min + dehydration in a hot oven at 53°C	PET jar and laminated film poach preserved better visual colour of the slices	Ansari *et al.* (2020)
Sealed polyethylene bags; sealed polypropylene bags	Sweet peppers pod	Storage for 28 days at 8°C after dipping in hot water at 50°C for 3 min and 55°C for 1 min	Polypropylene bags exhibited superior preservation of appearance, carotenoids, and ascorbic acid regardless of the temperature used for dipping	Said *et al*. (2013)
Polypropylene; polyvinyl chloride plastic film	'Yalova Charleston' Pepper	In normal atmosphere and MAP storage using 35µ PP and 35µ PVC at 7°C and 90±5% RH	Samples were successfully stored for 30 days using 35µ PP at 7°C and 90±5% RH	Akbudak (2008)
Polyethylene bag; jute sac; banana leaf; refrigerator; ambient condition; evaporative cooling system	Sweet pepperpod	Freshsealing	Combining an evaporative cooling system with a polythene bag proved to be a more effective storage method	Garuba *et al*. (2022)

Variety and breeding technologies

Developing breeding programs that effectively preserve the vibrant red color of peppers throughout the drying and processing stages plays a pivotal role in enhancing the appeal and desirability of red peppers. Furthermore, to prevent colour loss through breeding programs, the total carotenoid content of dried products from different cultivars should be compared and selected, and the cultivar with the highest total carotenoid content should be chosen (Paran and

Fallik, 2011). However, when comparing and selecting cultivars, one should consider not only the variety with the highest total carotenoid content but also the cultivar's carotenogenic capacity, which is manifested by a higher red-to-yellow isochromatic pigment fraction ratio (R/Y) and capsanthin to zeaxanthin ratio (Caps/Zeax) (Hornero-Méndez *et al.*, 2000).

Cultivar Mana had the highest total carotenoid content (13208 mg/kg dwt), but the lowest R/Y(1.25) and Caps/Zeax (3.38) ratios, so these parameters should be improved. Cultivar Negral, on the other hand, had a higher carotenoid content (8797 mg/kg wet), R/Y, and Caps/Zeax ratios, whereas cultivar Numex had the highest Caps/Zeax ratio (7.17) and lower total carotenoid content and should be improved by crossbreeding with Mana, which has a higher total carotenoid content (Hornero-Méndez *et al.*, 2000). Additionally, the capsaicin, heat level, and colour quality of known pepper genotypes revealed that genotypes have a significant effect on capsaicin and colour values. Furthermore, breeders should consider disease resistance as well as drying characteristics of peppers, as some genotypes are not suitable for drying, with increased pungency and colour loss during the drying process (Arpaci *et al.*, 2020). Gnayfeed *et al.* (2001) also reported that K-V2 variety had also the highest total carotenoids followed by F-03, SZ-178 and Cseresezenye, respectively.

The studies also revealed that the maturity stage has a significant impact on red pepper colour, with Candida red paprika having higher total carotenoids, b*, and L* values than Candida yellow paprika (Kim *et al.*, 2015). Different varieties considered for the study showed the same ripening-dependent pattern of b-carotene content increase, with the highest b-carotene content observed in the ripe red stage (Bhandari *et al.*, 2013). The total carotenoid content also decreased from 7966 to 1701 g/g in the break, faint red, and deep red stored pepper after three months, with faint red having the highest carotenoid content (Markus *et al.*, 1999). In general, breeding technologies, and varieties have a significant impact on the colour and other bioactive compounds of hot red pepper and should be considered in the management of red pepper colour loss (Martínez-Ispizua *et al.*, 2021).

6. Conclusions

In conclusion, the findings of this review paper indicated that colour fading hot red pepper is one of the major challenges in pepper production worldwide, which is highly determined by many factors such as genotype, maturity stage, exposure time to sunlight, higher temperature, relative humidity, moisture content and water activity, and oxygen during drying, processing techniques, storage methods, and product particle size. There is a need for significant effort to raise community awareness, engage stakeholders, and prioritize scientific research in order to address the inadequate attention given to preventing the fading of the bright red color in hot peppers. The use of light- and oxygen-permeable packaging materials, high-temperature drying, prolonged storage of the powder at high temperatures, and early and/or delayed harvesting were all noted in our review paper as contributing factors to the typical colour loss of red hot pepper powder. Based on the consulted literature, it is vital to employ techniques like low temperature drying, water blanching, and chemical dipping before the drying process in order to minimize color loss in red hot pepper powder. Furthermore, storing the peppers at a low temperature of 5°C, using packaging materials that are resistant to light, moisture, and air, and ensuring that the peppers are harvested at the appropriate maturity level are also essential steps to be taken in consideration.

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Review paper

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Plant biostimulants in ornamentals: Enhancing growth and stress tolerance

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Abstract: Researchers have recently sought a comprehensive strategy to reduce the harmful effects of synthetic chemicals in agricultural production to improve productivity and quality. Biostimulants benefit plants by protecting soil and water resources and eliminating adverse environmental effects from pesticides and chemical fertilizers. Plant biostimulants, also called bioactivators, are becoming increasingly well-liked in the agricultural sector due to their capacity to boost a plant's growth rate and increase its resistance to stress. Biostimulants are frequently used because they can increase crop quality, nutrient assimilation, growth rate, and stress tolerance. This article thoroughly examines biostimulants' effects on ornamental plants, concentrating on their ability to withstand environmental stressors. Prior studies have demonstrated that a combination of non-pathogenic microbes, protein hydrolysates, humic and fulvic acids, and algal extracts benefits ornamental plants. This review's main aims are biostimulants' effects on raising agricultural yield, enhancing nutrient uptake, enhancing photosynthesis, and shielding plants from biotic and abiotic stress. The role of biostimulants in more resilient and sustainable farming practices is also covered.

1. Introduction

The ornamental industry contributes significantly to both the global economy and agribusiness. It covers various activities, such as cultivating, distributing, and consuming flowers and attractive plants. The ornamental sector's economic worth, environmental benefits, and cultural relevance

demonstrate its significance. Worldwide, ornamental plant and flower production and trade produce significant money (Reis *et al.*, 2020; Krigas *et al.*, 2021). Employment opportunities are available in the sector, especially for small and family-run firms (Reis *et al.*, 2020). Additionally, the ornamental sector improves the aesthetic appeal of parks, gardens, and public places, contributing to the beauty of urban and natural landscapes (Rocha *et al.*, 2022). Additionally, ornamental plants have crucial environmental responsibilities as biological filters, wildlife habitats, and environmental purification agents (Rocha *et al.*, 2022). Ongoing innovation and developments in technology characterize the decorative industry. Digital tools and sensors are used in precision agriculture to monitor plant health, maximize resource utilization, and increase output efficiency. With the help of these technologies, producers can make better decisions, lessen their influence on the environment, and improve the quality of their decorative plants (Traversari *et al.*, 2021).

Plant biostimulants have drawn much interest in agriculture as a sustainable way to promote plant growth and raise crop output. Biostimulants containing organic materials and microbes have been discovered to improve stress tolerance, boost nutrient intake, and encourage growth crops (Parađiković *et al.*, 2019). Biostimulants present intriguing prospects to strengthen growth, quality, and stress tolerance in the context of ornamental plants, consequently enhancing the overall productivity of ornamental crops. Biostimulants can come from various sources, including non-pathogenic microorganisms, humic and fulvic acids, protein hydrolysates and algal extracts. These biostimulants have been shown to benefit plant growth in several ways, including higher yield, improved nutrient uptake and utilization, increased photosynthetic activity, and resistance to biotic and abiotic stresses (Calvo *et al.*, 2014). For biostimulants to be used effectively in horticulture, it is essential to understand the mechanisms underlying their impacts on ornamental plants. To thoroughly review the most recent studies on using biostimulants in cultivating ornamental plants, this study will examine their most recent findings. It will discuss the various biostimulants and how they affect ornamental crops' development and stress resilience. The evaluation will also examine how biostimulants might support resilient and sustainable horticulture.

2. Types of plant biostimulants

Microbial inoculants, such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR), are advantageous microorganisms that can boost nutrient uptake and increase stress tolerance in plants (Calvo *et al.*, 2014; González-González *et al.*, 2020). These biostimulants form symbiotic interactions with plants, which have several benefits, including increasing soil structure, nutrient availability, and plant defence systems. Organic molecules known as humic acids are produced when organic matter breaks down and are prized for their capacity to improve soil fertility, nutrient availability, and overall plant growth (Calvo *et al.*, 2014). They aid in forming roots, make nutrient absorption easier, and increase plant metabolism (Yakhin *et al.*, 2017). Fulvic acids, which are related to humic acids but have smaller molecules and better solubility, work in a manner comparable to that of humic acids. They help increase nutrient availability, root growth, and plant growth (Calvo *et al.*, 2014). Protein hydrolysates contain amino acids and peptides that help plants develop, absorb nutrients, and withstand stress. Amino acids are also created when proteins are broken down enzymatically (Calvo *et al.*, 2014; Ivankov *et al.*, 2021). These biostimulants increase plant vitality, encourage photosynthetic activity, and aid in root development. In agriculture, seaweed extracts, especially those from species like Ascophyllum nodosum, are frequently utilized as biostimulants (Calvo *et al.*, 2014; De Saeger *et al.*, 2019). Just a few of the bioactive components discovered in seaweed extracts that aid plants in growing, better-absorbing nutrients, and withstanding stress include hormones, polysaccharides, betaines, and phenolic compounds (De Saeger *et al.*, 2019; Boukhari *et al.*, 2020).

3. Molecular and physiological mechanism of plant biostimulants

Numerous studies have focused on the molecular and physiological processes of plant biostimulants. Several processes and routes are involved in how biostimulants positively affect plant growth, stress tolerance, and overall performance. One typical mechanism is the stimulation of crucial enzymes in nitrogen metabolism and the induction of hormone-like activity, such as auxin and gibberellin.

Biostimulants can modify a plant's root system and enhance its nutritional status. Increasing root biomass, density, and lateral root branching improves nutrient intake, assimilation, and translocation (Caruso *et al.*, 2019). It has been found that biostimulants can modulate phytohormones such as auxins, cytokinins, and gibberellins to affect plant growth and development. Additionally, they can boost photosynthesis, reduce aging, boost nutrition and water intake, and activate genes that defend against abiotic threats (Yakhin *et al.*, 2017). The metabolic changes caused by biostimulants have been better understood because of metabolomic studies. For instance, it has been shown that utilizing microbial biostimulants impacts metabolism, increasing the levels of tricarboxylic acid (TCA) intermediates, altering the amounts of amino acids, and changing the composition of phenolics and lipids. With these metabolic changes, plants are drought-resistant, have better defensive preconditioning, and promote growth. Additionally, biostimulants can modify redox homeostasis, strengthen plant cell walls, regulate osmoregulation, boost energy production, and remodel membranes, making plants more resilient and stress-tolerant (Nephali *et al.*, 2021). Plant biostimulants' molecular and physiological mechanisms involve several interactions and processes that enhance plant growth, stress tolerance, and overall performance. These techniques also control enzyme activity, metabolic adjustments, hormone-like effects, and the activation of genes and pathways related to development, stress response, and nutrient intake.

4. Mechanisms of action of plant biostimulants

The mechanisms of action of plant biostimulants are still not fully understood due to the complex nature of the raw materials used and the fact that the biostimulant products contain a variety of component mixtures (Yakhin *et al.*, 2017). However, research has shed light on some mechanisms that underlie specific biostimulants' action. Protein hydrolysate-based biostimulants have been discovered to promote plant development and mitigate the effects of abiotic stresses (Sorrentino *et al.*, 2021). The precise mechanisms of action of protein hydrolysates remain unknown. However, research has shown that they can affect specific metabolic pathways, including those involved in amino acid metabolism and phenolic chemical synthesis, enhancing plant growth and stress tolerance (Sorrentino *et al.*, 2021; Zhang *et al.*, 2023). The advantages of humic substances, such as humic and fulvic acids, have long been recognized in soil fertility and plant growth (Rouphael and Colla, 2018). The biostimulatory effects of humic chemicals are attributed to several mechanisms, including improved soil structure, increased nutrient availability, and nitrate assimilation (Rouphael and Colla, 2018). Microbial biostimulants, such as plant growth-promoting rhizobacteria (PGPR), have increased plant growth, nitrogen uptake, and stress tolerance (Kaushal *et al.*, 2023). Secondary metabolites, hormones, organic acids, and enzymes produced by microbial biostimulants help plant growth and activate defence mechanisms (Ma *et al.*, 2022; Kaushal *et al.*, 2023). Algal extracts and products made from seaweed have also been discovered to support plant development and stress tolerance (Mannino *et al.*, 2020). These biostimulants' possible modes of action include altering phytohormones, increasing photosynthetic activity, and inducing defensive responses (Yakhin *et al.*, 2017; Mannino *et al.*, 2020). Remembering that the type of plant and particular product can impact the biostimulant's mode of action is crucial. Mixing different biostimulants may have positive, negative, or cumulative effects (Giordano *et al.*, 2020). More research is needed to fully understand the biostimulants' mechanisms of action and maximize their use in sustainable farming practices.

5. Effects of plant biostimulants on growth and development

Improved growth and yield

Biostimulants have been proven to improve plant growth and raise agricultural production. Some examples are increased plant height, biomass buildup, and fruit production (Ertani *et al.,* 2014; Francesca *et al.,* 2020). Biostimulants can stimulate all elements of plant growth, such as root formation, shoot growth, and general vigour (Kocira *et al.*, 2020). Plant growth (germination rate, leaf area, chlorophyll and protein content, nitrogen content, root and stem development, root and stem weight biomass, yield, and drought resistance) has been shown to benefit from the application of biostimulants. Additionally, biostimulants can help with seed germination, increase crop production, and natural soil fertility,

and decrease pollutant toxicity. These are just a few of the positive benefits of biostimulants.

Enhanced nutrient uptake and utilization

Plant biostimulants can enhance a plant's capacity to take in and utilize nutrients. Increasing nutrient assimilation and absorption can improve nutritional status and resource utilization (Paradikovic *et al.*, 2019; Mohamed *et al.*, 2021). Improved plant nutrition and general development may result from this.Plant development and health are impacted both directly and indirectly by bacteria having PGPR properties. By fixing nitrogen, aiding in the production of plant hormones like auxin, cytokinin, and gibberellin, promoting the uptake of iron and similar trace elements through the production of bacterial siderophores, dissolving mineral and organic phosphates, and converting other nutrients into forms that the plant can easily absorb, these bacteria can directly affect the growth of plants. Or, as previously indicated, they are known to indirectly encourage plant development by reducing elements that cause plant disease (Dobbelaere *et al.*, 2003; Çakmakçı *et al.*, 2006).

Activation of metabolic pathways

Biostimulants can activate many plants' metabolic processes, promoting better growth and development. They can alter the synthesis and metabolism of plant hormones such as auxins, cytokinins, and gibberellins, which are crucial for regulating plant growth (Nephali *et al.*, 2021; Sorrentino *et al.*, 2021). Although the effect of biostimulants on metabolic processes has not been fully revealed, biostimulant contents may be associated with the synthesis of molecules or precursors involved in these processes. For instance, protein hydrolisates may function as regulators for plant growth, as they contain short-chain peptides, and certain amino acids, such as phenylalanine, have been reported to increase the production of endogenous auxin by functioning as signaling molecules (Raguraj *et al.*, 2022). Additionally, biostimulants, such as phenolic compounds, may affect the production of secondary metabolites that enhance plant defence and stress tolerance (Nephali *et al.*, 2021).

Stress tolerance and resistance

One of the main benefits of biostimulants is their ability to boost plant stress tolerance. They can make plants more resilient to abiotic stresses such as heat, salt, and drought (Oosten *et al.*, 2017; Nephali *et al.*, 2021). Furthermore, biostimulants can increase resistance to biotic stresses like pests and illnesses (Godlewska *et al.*, 2021). They can strengthen the immune system, activate defence systems, and enhance the plant's overall stress response.

Improved fruit quality and nutritional value

Biostimulants have been shown to enhance the nutritional value and quality of fruits and vegetables. They can increase the amount of bioactive components in crops, such as vitamins, phenolic compounds, and antioxidants, enhancing their nutritional value and health-promoting properties (Francesca *et al.*, 2020; Godlewska *et al.*, 2021). Biostimulants can also improve the sensory characteristics of fruits, such as color, flavor, and shelf life.

Plant biostimulants improve a plant's capacity to grow, develop, and tolerate stress. They can promote crops' nutritional content and quality, nutrient uptake, metabolic activity, stress resistance, and other processes (Calvo *et al.*, 2014). Because of these effects, biostimulants are valuable tools in sustainable agriculture for increasing crop productivity and resilience.

Enhancing stress tolerance in ornamental plants

With positive findings, using plant biostimulants has increased the stress tolerance of ornamental plants. Using biostimulants, abiotic stressors such as drought, heat, and water scarcity can decrease and improve plant performance (Toscano *et al.*, 2019; Nephali *et al.*, 2021). By triggering antioxidant defence mechanisms, controlling reactive oxygen species (ROS) metabolism, and enhancing membrane integrity, they can improve plant responses to stress (Nephali *et al.*, 2020; Hasanuzzaman *et al.*, 2021). Studies have shown that biostimulants can help ornamental plants flower earlier, produce more flowers, and accumulate biomass (Toscano *et al.*, 2019). Furthermore, it has been discovered that biostimulants increase the tolerance of ornamental plants to drought stress, leading to more significant growth and survival rates (Toscano *et al.*, 2019; Leotta *et al.*, 2023). Promoting root growth, enhancing nutrition and water availability, and managing stomatal function can increase the plant's resistance to water scarcity (Clercq *et al.*, 2023; Leotta *et al.*, 2023). Some biostimulants have

examined the resilience of ornamental plants. Studies, for example, have demonstrated that protein-rich seaweed extracts, such as *Chondrus crispus*, serve as biostimulants and improve drought tolerance in tomato plants (Domingo *et al.*, 2023). Seaweed extracts are known to increase several crops' resiliency to stress, especially ornamentals (Francesca *et al.*, 2020; Clercq *et al.*, 2023). It has also been investigated whether microbial biostimulants, such as rhizobacteria that promote plant development, can improve ornamental plants' resilience to stress (Nephali *et al.*, 2021).

Plant biostimulants throughout cultivation can considerably improve ornamental plants' performance and stress tolerance. Utilizing biostimulants enables plants to respond to abiotic challenges, develop, and grow more effectively, which enhances the quality of attractive crops. More investigation is required to comprehend the mechanisms of action and to improve administration strategies and biostimulant dosages for diverse species of ornamental plants.

6. Application methods and dosages of plant biostimulants

Application methods

Before planting, seeds can be treated with biostimulants to improve germination, seedling vigour, and early growth (Paradikovic *et al.*, 2019). Foliar application is another one to boost nutrient intake, spur growth, and increase stress tolerance; biostimulants can be sprayed into the leaves of ornamental plants (Mannino *et al.*, 2020; Cristiano and De Lucia, 2021). Spraying biostimulants on the substrate is another application method to promote root development, nutrient uptake, and overall plant growth. Biostimulants can be sprayed on the substrate or growing media surrounding the roots of ornamental plants (Lorenzo *et al.*, 2018; Santos *et al.*, 2019). Irrigation systems can also be used to apply biostimulants, which will then be delivered right to the root zone of ornamental plants (Paradikovic *et al.*, 2019).

Dosages

Different biostimulants may require different dosages depending on the product, plant type, and growth stage. Various research has evaluated a range of biostimulant application doses or rates. For instance, a biopolymer-based biostimulant was used in a study on melon plants at rates of 0.06, 0.12, 0.24, or 0.48 mL per plant (Lorenzo *et al.*, 2018). Compared to lower concentrations, the application at 0.12 and 0.24 mL per plant led to better plant growth and biomass. An animal-derived biostimulant was administered to potted snapdragon plants in a different investigation at concentrations of 0, 0.1, or 0.2 g L^{-1} (Cristiano *et al.*, 2018). The biostimulant application at both doses significantly increased plant height, shoot length, leaf area, flower number, and aboveground dry weight compared to the control. Depending on the particular needs of the ornamental plant species and the desired results, the dosage and frequency of biostimulant application may need to be changed. It is significant to note that depending on the biostimulant product, plant species, environmental factors, and growth stage, the best application techniques and dosages of biostimulants may change. As a result, it is advised that site-specific studies be carried out and that the manufacturer's instructions for the biostimulant product be adehered to.

7. Biostimulant application in ornamental plants

Biostimulant applications have recently started to be in high demand in ornamental plants. Many studies have demonstrated that it naturally promotes rooting and plant growth as an alternative to commercial hormone uses. In some ornamental plants with high commercial value, it has been stated that PGPR applications positively affect both rooting promotion and agronomic properties.Studies have been carried out on ornamental plants belonging to different families Asteraceace (*Chrysanthemum, Dahlia, Zinna*) and Geraniaceae (Göre and Altın, 2006); *Iridaceae* (Gladiolus) and *Oleaceae* (Jasmine) (Damodaran *et al.*, 2014) and *Solanaceae* (Petunia) (Hoda and Mona, 2014).

In the studies, it has been determined that biostimulants positively affect rooting in ornamental plants.It has been stated that *P. fluorescens* bacterial strain in *Zinnia* (Yuen and Schroth, 1986); *Azospirillum brasilense* strains in Photinia (Larraburu *et al.*, 2007); *Agrobacterium rubi* and *Serratia liguefaciens* strains in *Forsythia intermedia* (Kır, 2010); *Bacillus megaterium* and *Pseudomonas flourescens* strains in *Rosa canina* (Kınık and Çelikel, 2017); *Bacillus subtilus* strain in *Ficus benjamina* L.

(Sezen *et al.,* 2014); different *Bacillius* strains in *Kalanchoe blossfeldiana* (Dalda-Sekerci and Unlu, 2023) have been shown to promote rooting.

The effects of biostimulants on the vegetative characteristics, flowering status, duration of flowering, and tuber formation of ornamental plants were also reported.Positive effects of biostimulants on agronomic properties were reported in anthurium (Padmadevi *et al.*, 2004); chrysanthemum and dahlia (Gore and Altın, 2006); geranium (Mishra *et al.*, 2010), tulip (Parlakova, 2014), gladiolus (Damodaran *et al.*, 2014), jasmine (Jayamma *et al.*, 2014); in petunia (Hoda and Mona, 2014); in the poinsettia (Parlakova Karagöz, 2018); and cyclamen (Girgin and Sezen, 2021) summary of reported beneficial effects of different biostimulant types and application techniques given Table 1.

8. Regulatory aspects and future perspectives

Clear definitions and rules controlling plant biostimulants are required because the regulatory framework for these substances is currently developing (Yakhin *et al.*, 2017). To promote the legalization of biostimulants and ensure their efficacy and safety, the European Union (EU) has tried to regulate them (Farkas *et al.*, 2022). Beyond necessary nutrients or plant growth regulators, the concept of biostimulants emphasizes their capacity to increase plant productivity through the unique features of their constituents (Yakhin *et al.*, 2017). Biostimulants must be regulated to maintain product quality, efficacy, and consumer confidence and support ecologically friendly and sustainable agricultural practices (Farkas *et al.*, 2022). The ability of plants to function better under stress and produce higher-quality plants overall is greatly enhanced by using biostimulants in the cultivation of ornamental plants (Farkas *et al.*, 2022). Additional research is required to comprehend further the mechanisms of action and the impacts of various biostimulants on ornamental plants (Nephali *et al.*, 2020). For the efficient and long-term use of biostimulants in agriculture, creating a science-based biostimulant industry and sound regulations are essential (Yakhin *et al.*, 2017). Application strategies, doses, and timing of biostimulant treatments in ornamental plants can all be improved to increase efficacy (Farkas *et al.*, 2022).

Combining biostimulants with environmentally friendly agriculture techniques like organic fertilizers and integrated pest management can produce more robust and sustainable systems for growing decorative plants (Farkas *et al.*, 2022). In conclusion, efforts are being made to assure the safety, effectiveness, and appropriate use of plant biostimulants in cultivating ornamental plants. Biostimulants' prospects include more study, method optimization, and integration with other sustainable practices to improve plant performance and resilience.

9. Conclusions

As a result, plant biostimulants have demonstrated significant promise for improving ornamental plants' growth and stress tolerance. The research that has been evaluated has shown that biostimulants can boost ornamental crop performance, yield, nutrient uptake, and stress tolerance.

The mechanisms of action of biostimulants include stimulating antioxidant defence systems, activating metabolic pathways, and modifying plant hormone levels. Using biostimulants to develop ornamental plants provides opportunities for resilient and sustainable horticulture techniques. By lowering the need for artificial fertilizers and pesticides, biostimulants can aid in developing more effective and ecologically friendly agricultural methods. They can also raise ornamental plants' aesthetic appeal and quality, increasing their marketability.

More study and development are required to maximize the utilization of biostimulants in the growth of ornamental plants. This includes examining the precise results of various biostimulant formulations and biostimulant kinds on various species of ornamental plants. The best biostimulant application strategies, doses, and timing for various growth phases and environmental circumstances also require further research. Specific definitions and regulations are required to guarantee product quality, efficacy, and consumer confidence. Regulatory aspects of biostimulants are also evolving. For biostimulants to be used effectively and sustainably in producing ornamental plants, a science-based biostimulant industry, and good laws are essential. To sum up, plant biostimulants have much potential to improve ornamental plants'

Table 1 - Different biostimulant agents and beneficial effects on ornamental plants

Biostimulant type	Applied species	Application	Beneficial effects	References
Spirulina and Klamath algae	*Portulaca grandiflora*	Mixing with the growing substrate	To improve seed germination, plant growth and flowering	Prisa (2019)
Animal-derived PH (hydrolysis of proteins from erythrocytes	*Antirrhinum majus* L.	0.1 and 0.2 gL^{-1} foliar spray and root drenching (150 ml/plant)	To increase plant morphological and qualitative traits, leaf and root-N content, photosynthetic rate, transpiration rate, and stomatal conductance	Cristiano *et al.* (2018)
Seaweed extract (*Ascophyllum nodosum*)	*Helianthus annus* L. cv. Pleno Sol	0, 5, 10 or 15-mLL^{-1} of seaweed extract (60 mL spray treatment during seed germination)	To enhances seed germination and seedlings development	Santos *et al.* (2019)
Animal derived PH (Hicure®)	*Dianthus caryophyllus* L.	Drenching with biostimulant concentrations of 2.0, 2.5 and 3.0 L ha^{-1}	Improvement of flower quality, such as stem length and flower head size	Niyokuri *et al.* (2017)
Chitosan nanoparticles	*Rosa hybrida* cv. Black magic	Applying as vase solution with the concentration of 5, 10, 15 mg L^{-1}	Reduction microbial growth in vase solution. Increase phenolics, total flavonoids, and amount of anthocyanin in treated petals and vase life of the flowers	Seyed Hajizadeh *et al.* (2023)
Plant derived Protein hydrolysates (PH)(Trainer® and Vegamin©)	*Chrysanthemum morifolium* cv. Pinacolada and Radost	Spraying the whole plant at the recommended concentration by the companies	Overall action; improved the status of plants, stimulating stem elongation and the apical flower diameter	Carillo *et al.* (2022)
Animal derived PH (Hydrostim®)	Petunia × *hybrida* Hort. cv. Potuniaand Dunnen	0, 0.1, 0.2 g L^{-1}foliar spray and root drenching	Enhance visual quality of the plants (increase leaves and flower numbers, leaf area, dry weight, shoots, flowers, and leaf fresh weight	Cristiano and De Lucia (2021)
Cyanobacterial hydrolysate (*Arthrospira platensis*)	*Petunia x hybrida*	5 g L^{-1} foliar spraying weekly under salinity stress conditions	Hydrolysate mitigates the negative effect of NaCl on *Petunia* x *hybrida* crops at an EC of 3.0 dS m^{-1}	Bayona-Marcillo *et al.* (2020)
Fermented alfalfa brown juice	Tagetes patula L. cv. Csemő	0.5%, 1.0%, 2.5%, 5.0%, or 10% of fermented alfalfa brown juice	0.5% fermented BJ improved seed germination root and shoot length, root and shoot dry mass and the number of leaves	Barna *et al.* (2021)
Vegetal extract and PH (Radifarm®)	Viola tricolor var. *hortensis* DC.	Applying to the plant rhizosphere at 0.3% solution	Improvement seedling quality and morphological parameters	Zeljković *et al.* (2021).
Microalgae (*Arthrospira platensis*) hydrolysate	*Pelargonium hortorum* L.H. Bailey	Foliar spraying at the 5 g/L concentration with 150 mg/L silicon	Stimulation root, shoot, leaf, and flower formation under salinity stress.	Tejada-Ruiz *et al.* (2020)

Table 1 - Different biostimulant agents and beneficial effects on ornamental plants

Biostimulant type	Applied species	Application	Beneficial effects	References
Seaweed extract (Acadian Seaplants™)	*Tagetes erecta*	Spraying to seeds daily with 70 ml solution at the 0, 5, 10, 15 ml L^{-1} concentrations	Enhancing seed germination and seedling growth and development (optimum concentration was 15 ml/L)	Tavares *et al.* (2020)
Microbial biostimulant (*Trichoderma sperellumand T. harzianum)*	*Passiflora caerulea*	Foliar application with spores at the concentration of 10^6 and 10^8 cfu mL^{-1}	Increase number and size of chloroplasts, improved plant physiology characteristics, and an increase yield	Şesan *et al.* (2020)
Vegetal extract (*Moringa oleifera*)	*Gladiolus grandiflorus*	5% (*v/v*) aqueous extract alone or in combination with 50 mg/L salicylic acid or gibberellic acid	Increase the yield and quality of cut spikes, prolong the vase life.	Zulfiqar *et al.* (2020)
Nitrophenolate based biostimulant (Atonik®)	*Calendula officinalis*	Plants irrigated by 3000 ppm saline water and sprayedwith 2 mlL^{-1}Atonik (0.196 Ug^{-1})	Tolerate the salt stress and promoted flowering growth	El-Ziat and Swaefy (2019)
Arbuscular mycorrhizal fungi (*Glomus mossease*)	*Gerbera jamesonii* cvs. Beaudine and Palm Beach	AMF spores were added to soil at a rate of 100 spore/1000 g of dried soil	AMF inoculation at seedling stage can significantly increase gerbera flower yield and longevity after harvesting (vase life)	Othman *et al.* (2022)
Arbuscular mycorrhizal fungi *(Funneli formis mosseae)*	*Hyacinths orientalis* L. Anna Marie	Approx. 1100 spores were added to 2 kg substrate with soils and sands (3:1, v/v)	Regulate early flowering and prolonging flowering time	Xie and Wu (2018)
Rhizobacteria (*Azospirillum brasilense*)	*Eustoma grandiflorum* (Raf) Shinn.	Inoculation with 106 and 107 cfu on seed surface at sowing	Improve morphological parameter of seedlings and lead to a shorter time production	Santos *et al.* (2022)
Humic acid and vermicompost	*Lavandula angustifoli*a L.	Adding vermicompost to the soil before planting, foliar application of humic acid	Increase flower yield and essential oil content, reduce the demand for chemical fertilizers	Sharafabad *et al.* (2022).
Humic acid	Lilium Oriental Hybrids *'Sorbonne'*	Adding 0.2, 2.0, and 20.0 mgL^{-1} humic acid directly to the medium	Promote *in vitro* bulblet growth increase in bulblet sucrose, total soluble sugar, and starch content	Wu *et al.* (2016)

growth, stress tolerance, and general performance. Future horticulture practices will be more resilient and sustainable thanks to the optimization of biostimulant use made possible by ongoing research and development in this area.

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Short note

Inter-annual and genotypic variation of morphological and physicochemical characters in Moroccan loquat (*Eriobotrya Japonica* Lindl.)

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Abstract: Plant development is constantly affected by biotic and abiotic factors, which influence their morphology and chemical composition. In this context, the evaluation of morphological and physicochemical variation of 35 loquat genotypes during two consecutive years, 2015 and 2016, were carried out. The results revealed a significant difference of the morphological and the physicochemical traits between the two years. Indeed, 2016 showed high values for fruit and leaf traits as well as the physicochemical parmeters, while 2015 recoreded the highest values for seeds traits. In addition, the ANOVA results showed a significant effect of genotype on the physicochemical parameters and the morphological characters, excluding the geometric diameter and spherical index of seeds. Regarding the effect of year, it was also significant on physicochemical parameters and morphological traits except the size and shape of fruit and the seed shape. For the genotype x year interaction effect, it was significant on all traits studied, with the exception of the traits relating to geometric diameter of fruit and seed plus the sphericity index of the seed. Thus, size and shape of fruit remained stable over these two consecutive years. The identification of stable traits presents a result that could be beneficial for breeding programs.

1. Introduction

Loquat (*Eriobotrya japonica* Lindil.), belonging to *Rosaceae* family, is an evergreen tree, native to China (Gariglio *et al.*, 2002). The world production of loquat is about 314,384 tons, 64% of this amount exhibited by China (Caballero and Fernández, 2003). In the Mediterranean region, loquat crops are highly developed, particularly in Spain and Turkey (Del

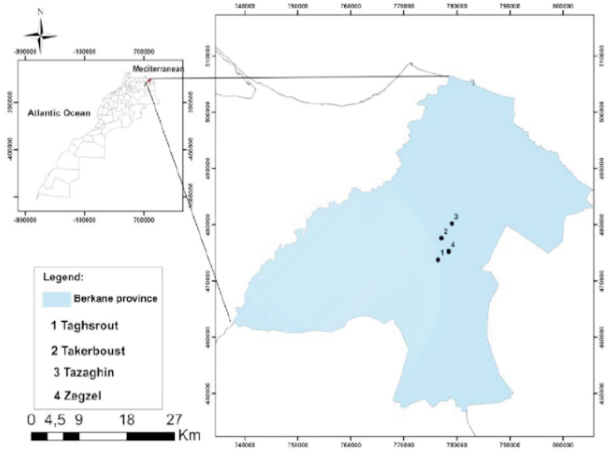
Mar Romero Escudero *et al*., 2011). Loquat cultivation is considered a commercial crop in some countries, while in others it is grown only in family orchards (Caballero and Fernández, 2003). In Morocco, this tree, with its yellow fruits, is planted as a commercial consumable crop as well as an ornamental crop (Hussain, 2011) in the regions of Fez-Meknes, Khemisset, Tetouan and in the region of Marrakech but it is localized especially in Berkanewith an area representing 85% of the national surface (Skiredj and El Macane, 2003). The Berkane region is mostly covered by loquat tree due to its mild and sunny microclimate and well-drained fertile soils (Rhomari, 2013). In 2021, the production of loquat in Berkane crossed 10,000 tons, with an improvement in the size of the fruits and the gustative quality of this excellent local product (Chellay, 2022).

The estimates of trait heritability were found to be relatively low to moderate (Jiwuba *et al.,* 2020). Ezenwaka *et al*. (2018) suggests that the combination of genotype and environmental effects greatly influenced trait expression. Indeed, phenotypic expression as well as observed variation in plant growth and development depend on both genetic background (G), environment (E) and their interaction (G × E) (Falconer and Mackay, 1996). A result, a clear understanding of G×E will provide a solid basis for identifying superior and stable genotypes in different environments (Zhang *et al*., 2010). The study of the effect of these factors on the variation of morphological and chemical parameters of plants, including loquat, is limited, but with climate change it has become an obligation. In the region of Berkane, the main producer of this fruit in Morocco, the temperature recorded a decrease, while the rainfall increased during 2015 and 2016 (Meteobleu, 2024). These observations encouraged to explore the effect of these changes on the morphological and physicochemical characteristics of 35 loquat genotypes during these two consecutive years. Indeed, high trait stability present one of the main challenges of plant breeding programs.

2. Materials and Methods

Plant material

In April 2015, a prospection was carried out in loquat plantations of Zegzel, Takerboust, Taghsrout and Tazaghin, belonging to the Berkane region, to identify the genotypes that will be involved in this study (Fig. 1). The choice of the genotypes based on a numerous agronomic and economic criteria, such as tardiness and earliness, shape, size and colorof fruit, shape of leaves as well as the good physical condition of tree. Indeed, a total of 10 mature, healthy fruits and 10 well-developed leaves were collected randomly during April and May of 2015 and 2016 from 35 adult and young trees.



Geographical origin	Distance from Berkane center (km)	Number of samples	rainfall (mm)		Temperatures (°C)	
			2015	2016	2015	2016
Tazaghin	8	4				
Takerboust	12	12				
Zegzel	16	10	282.9	352.8	11.8-26.1	12.2-25.7
Taghsrout	20	9				
Total		35				

Fig. 1 - Sampling sites of loquat genotypes studied and their geographic and climatic parameters.

Morphological analysis

A total of 17 characteristics related to leaves, fruits and seeds,listed in International Union for Plant Protection descriptor (UPOV, 1998), were investigated (Table 1). The weights were determined using an electronic balance with a sensitivity of 0.01 g, while the dimensions were measured using a digital caliper (Stainless Hardned) with a sensitivity of 0.01 mm. Leaf length measured as the distance from the apex to the base of the leaf, including the blade and petiole. Regarding the blade width, it is measured at the widest part of the leaf.

Physicochemical analysis

Soluble solid content, determined by a refractometer (ATAGO C.O. Ltd; Model PR-1). Briefly, a few drops of the juice of each genotype were placed on the prism of the equipment surface and the soluble solid content expressed in °Brix. The titratable acidity was measured by potentiometric titration using a standardized alkaline solution (Serrano *et al.,* 2003).

Table 1 - Fruit, seeds, leaf traits and physicochemical parameters analyzed

Traits	Code
Fruits	
Weight of the fruit (g)	FW
Geometric diameter of the fruit (mm)	GDF
Sphericity index of the fruit	SIF
Surface of the fruit (mm^2)	SF
Volume of the fruit (cm^3)	VF
Weight of flesh (g)	WF
Flesh ratio	FR
Seeds	
Average weight of the seed (g)	AWS
Geometric diameter of the seed (mm)	GDS
Sphericity index of the seed	SIS
Surface of the seed (mm^2)	SS
Volume of the seed (cm^3)	VS
Leaf	
Leaf length (cm)	LL
Blade length (cm)	BL
Blade width (cm)	BW
Petiole length (cm)	PL
Number of veins	NV
Physicochemical parameters	
Soluble solids content (°Brix)	
Titrable acidity (g/l malic acid)	
pH	

GDF= (Fruit length x Fruit width x Fruit thickness)$^{0.333}$;
SIF= GDF/Fruit length;
SF= π x GDF^2;
VF= (π/6) x GDF^3;
WF= FW-AWS;
RF= FW-AWS/FW;
GDS= (Seed length x Seed width x Seed thickness)$^{0.333}$;
SIS= GDS/Seed length;
SS= π x GDS2;
VS= (π/6) x GDS3;
NV were counted.

An amount of 10 ml of fruit juice was diluted in 50 ml of distilled water and then titrated with a 0.1 NaOH solution until pH 8.2 was reached. Titratable acidity is expressed per g malic acid L^{-1}. Concerning the pH values of the juice, it was measured using an electronic pH meter (PH211R, HANNA®) with three replicates for each sample.

Statistical analysis

Data obtained were subjected to statistical analysis using SAS software (SAS Institue Inc., 1988). Indeed, the two-way analysis of variance (ANOVA) tests was performed to determine the effect of genotype, year and genotype x year interaction on the morphological traits as well as the physicochemical parameters of genotypes. The comparison of means was performed by Duncan's test.

3. Results

The assessment of morphological and physicochemical characters of 35 loquat genotypes, from the Berkane region, revealed a significant difference between the two consecutive years (Table 2). In addition, a significant effects of genotype, year and their interaction on the most of the traits studied, were recorded (Table 3). In fact, the comparison of fruit values indicated a significant difference of fruits

Table 2 - Averages comparison of fruit, seed, leaf and physicochemical characteristics during 2015 and 2016

Traits	Years	
	2015	2016
Fruit		
Fruit weight	41.02 b	42.73 a
Geometric diameter of the fruit	42.28 a	41.58 a
Sphericity index of the fruit	0.86 a	0.87 a
Fruit surface	53.86 a	54.98 a
Fruit volume	37.96 a	39.03 a
Weight of the flesh	34.80 a	35.55 a
Flesh ratio	0.81 b	0.83 a
Seed		
Average weight of the seed	2.58 a	2.36 b
Geometric diameter of the seed	15.11 a	14.43 a
Sphericity index of the seed	0.71 a	0.72 a
Surface of the seed	7.23 a	6.36 b
Volume of the seed	1.85 a	1.54 b
Leaf		
Leaf length	22.47 b	23.7l a
Blade length	21.16 b	22.5l a
Blade width	6.46 b	7.34 a
Petiole length	1.3l a	1.20 b
Number of veins	0.41 b	0.43 a
Physicochemical characteristics		
Soluble solids content	7.17 b	9.77 a
Titrable acidity	5.69 b	8.09 a
pH	3.18 b	4.52 a

Table 3 - Genotype, year and their interaction effect on fruit, seed, leaf and physicochemical characters

Source of variation	ddl	Mean square	F-Value	Pr>F
Fruit				
Fruit weight				
Genotype	34	1746.70	22.03	<.0001
Year	1	512.50	6.47	0.0112
Genotype × year	34	472.97	5.97	<.0001
Error	630	79.27		
Geometric diameter of the fruit				
Genotype	34	495.17	2	0.0008
Year	1	84.38	0.34	0.5593
Genotype × year	34	308.47	1.25	0.1608
Error	630	247.26		
Sphericity index of the fruit				
Genotype	34	0.06	17.09	<.0001
Year	1	0.0006	0.17	0.6763
Genotype × year	34	0.01	3.09	<.0001
Error	630	0.003		
Fruit surface				
Genotype	34	1573.73	24.28	<.0001
Year	1	218.49	3.73	0.0668
Genotype × year	34	13125.09	5.96	<.0001
Error	630	64.81		
Fruit Volume				
Genotype	34	1682.73	22.39	<.0001
Year	1	198.99	2.65	0.1042
Genotype × year	34	421.05	5.60	<.0001
Error	630	75.14		
Weight of the flesh				
Genotype	34	877.43	19.18	<.0001
Year	1	65.57	1.43	0.2318
Genotype × year	34	218.82	4.78	<.0001
Error	452	45.74		
Flesh ratio				
Genotype	34	0.01	6.81	<.0001
Year	1	0.02	14.20	0.0002
Genotype × year	34	0.008	5.03	<.0001
Error	452	0.001		
Seed				
Average weight of the seed				
Genotype	34	2.13	5.95	<.0001
Year	1	5.43	15.18	0.0001
Genotype × year	34	1.31	3.68	<.0001
Error	451	0.35		
Geometric diameter of the seed				
Genotype	34	20.00	1.26	0.1563
Year	1	54.60	3.43	0.0647
Genotype × year	34	12.21	0.77	0.8266
Error	455	15.91		
Sphericity index of the seed				
Genotype	34	0.031	1.12	0.3
Year	1	0.017	0.62	0.4332
Genotype × year	34	0.022	0.80	0.7823
Error	455	0.028		
Seed surface				
Genotype	34	9.57	6.66	<.0001
Year	1	89.07	61.94	<.0001
Genotype × year	34	3.83	2.67	<.0001
Error	455	1.43		
Seed volume				
Genotype	34	1.26	6.75	<.0001
Year	1	11.57	61.70	<.0001
Genotype × year	34	0.51	273	<.0001
Error	455	0.18		
Leaf				
Length of the leaf				
Genotype	34	98.25	5.79	<.0001
Year	1	252.43	14.88	0.0001
Genotype × year	34	120.18	7.08	<.0001
Error	617	16.96		
Blade length				
Genotype	34	90.19	5.71	<.0001
Year	1	296.4	18.75	<.0001
Genotype × year	34	296.4	7.07	<.0001
Error	617	15.86		
Blade width				
Genotype	34	15.67	6.04	<.0001
Year	1	128.4	49.48	<.0001
Genotype × year	34	14.03	5.41	<.0001
Error	617	2.59		
Petiole length				
Genotype	34	0.59	8.64	<.0001
Year	1	1.77	25.95	<.0001
Genotype × year	34	0.33	4.51	<.0001
Error	617	0.06		
Number of veins				
Genotype	34	0.071	8.84	<.0001
Year	1	0.075	8.05	0.0047
Genotype × year	34	0.076	8.30	<.0001
Error	617	0.008		
Physicochemical parameters				
Soluble solid content (°Brix)				
Genotype	36	12.45	19.74	<.0001
Year	1	201.55	319.42	<.0001
Genotype × year	31	5.76	9.14	<.0001
Error	71	0.63		
Acidity				
Genotype	36	13.91	5.82	<.0001
Year	1	205.51	85.89	<.0001
Genotype × year	31	4.92	2.06	0.0064
Error	71	2.39		
pH				
Genotype	36	0.33	14.17	<.0001
Year	1	59.88	2506.15	<.0001
Genotype × year	31	0.19	8.21	<.0001
Error	71	0.02		

weight and flesh ratio among two years. These traits showed high values in 2016 with 42.73 g and 0.83 respectively, in comparison to those obtained in 2015 with 41.02 and 0.81 respectively. The remaining traits are much more stable. Regarding the effects of

genotype, year and their interaction on the fruit traits, the results showed high significant effect of genotype on all traits ($p<0.0001$), while the year effect was significant only on fruit weight and flesh ration ($p<0.05$). Whereas, the effect of genotype × year interaction was very significant on all traits analyzed ($p<0.0001$), except on geometric diameter of fruits.

For the seed results, in 2015 the averages of weight, surface and volume were higher (2.58 g, 7.23 mm^2 and 1.85 cm^3 respectively) than those obtained in 2016 (2.36 g, 6.36 mm^2 and 1.54 cm^3 respectively. This result was confirmed by the significant effects of genotype, year and their interaction on average weight, surface and volume of the seed ($p<0.0001$). The rest of traits such as geometric diameter and sphericity index of seed seems to be not affected by this factor. As results, there is a significant combined effect between genotypes, year and genotype × year interaction on the weight, volume and as well as seed surface.

Moreover, the average values of leaf length, blade length, blade width and number of veins were higher in 2016 (23.71 cm, 22.5 cm, 7.43 cm, and 0.43 cm respectively) compared to those obtained in 2015 (22.47 cm, 21.16 cm, 6.46 cm, and 0.43 cm respectively). While, the petiole length recorded the highest value in 2015 (1.31 cm). In addition, the results of ANOVA showed highly significant effects of genotype, year and genotype × year interaction on all leaf traits studied ($p<0.001$).

Furthermore, the comparison of the soluble solids content, acidity and pH results for two years indicated that the values of these parameters were higher in 2016 (9.77°Brix, 8.09 g/l malic acid, 4.52) than those registered in 2015 (7.17°Brix, 5.69 g/l malic acid, 3.18). Moreover, the statistical analysis showed high significant effects of genotype, year and genotype × year interaction on these parameters ($p <0.0001$).

4. Discussion and Conclusions

The comparison of the averages of the studied traits during two consecutive years and the evaluation of the effects of genotype, environment and their interactions allow to measure the stability of the characterscanbe integratedin breeding programs (Ebdon and Gauch, 2002). In this regard, the present study revealed a significant variation ofmorphological and physicochemical traits of 35 loquat genotypesduring 2015 and 2016 as well as the magnitude of the inter-annual variation of the studied traits depending on the genotype. Some traits are stable, while others showeda significant variationfrom one year to the next. Effectively, the average values of fruit weight and flesh ratio parameters were different between 2015 and 2016 which showed the highest values of these traits. Similarly, Elsabagh and Haeikl (2012) recorded a significant difference of fruit weight of four Egyptian loquats during 2011 and 2012. In loquat, it has been reported that fruit weight depends mainly on genotype (Gariglio *et al*., 2001; Lin *et al*., 1999) and the cultivation conditions, which present a notable effect on the characteristics of the fruit (Cuevas *et al*., 2012). In fact, the increase of fruit weight can be attributed to the amount of rainfall and low temperatures recorded during the 2016 in comparaison with 2015 in Berkane region. In addition,the tree load had a negative effect on fruit size, so that the proportion of large fruits increased as the number of fruits per tree decreased (Mahhou *et al*., 2006). In addition, these good resultwas due to a program initiated during 2016. This program provided a supplementary training for farmers and purchasing technical equipment, packaging supplies and equipment for a refrigeration unit in order to develop and improve the loquat crop (Chellay, 2022). Concerning the seed results, the average weight, surface and volume of the seed, were superior in 2015 than values obtained in 2016, with a significant influence of genotype, year and genotype × year interaction. In fact, the results revealed a significant combined effect between genotype, year and their interaction on seed weight, seed volume and seed area, but its effect was not significant on geometric diameter and seed sphericity index. This finding is in agreement with that reported by Elsabagh and Haeikl (2012), which found a significant difference among 2011 and 2012 of the seed weight of four Egyptian loquat trees. This result could be due to the amount of rainfallrecorded in 2015, compared to 2016 which influenced the plants growth in the Berkane region (Zejly, 2016). Whereas,severe water stress during seed fill of soybean plantscaused their inability to regulate seed number and changing the weight distribution of the seeds to a higher proportion of small seeds. As a consequence, a greater number of small seeds (Dornbos and Mullen, 1991). For the leaf traits, the year 2016 is characterized by higher values of leaf length, blade length, blade width and number of

veins compared to those obtained in 2015. In addition, the results revealed a highly significant effects of genotype, year and genotype × year interaction on all the leaf variables studied. In cassava, the percentage of variation due to environment was higher than the percentage of variation due to genotype for leaf retention, indicating that the environment strongly influenced the expression of this trait (Jiwuba *et al*., 2020). Also, the phenotypic plasticity for leaf size, specific leaf area, and leaf level of hybrid poplars are modulated by a variety of environmental factors, including light, nutrient availability, and water availability (Toillon *et al.,* 2013). Regarding the sugar content, acidity and pH, which were registered during the year 2016 are superior to those recordedin 2015. This result is reinforced by the strong effect recorded of genotype, year and their interactions on these parameters. In the Egyptian loquat, the same result was obtained by Elsabagh and Haeikl (2012). The authors observed a significant effect of the year on acidity and sugar content of some loquat varieties during 2011 and 2012. The tree charge influences soluble solids content, which increased as the number of fruits per tree decreased. Thus, the soluble solids content of the fruit increased with fruit size (Mahhou *et al*., 2006).

According to the results obtained, the variation of morphological and physicochemical traits analyzedwas very importantwith an high effect of genotype factor. Nevertheless, fruit size and shape, which are the most important economic criteria, were found to be stable over these two consecutive years. These finding should be considered in breeding programs for more effective control of fruit quality. Further research is needed to control the impact of these factors on the stability of the selected plant. Moreover, a future research should also investigate the effect of these factors on biochemical composition.

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