



Influence of Irrigation Intervals and Exogenous Applications of Brassinolide and Chitosan on Production and Quality Attributes of Tomato

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ABSTRACT

The research investigated the varying irrigation intervals and concentrations of Brassinolide and Chitosan for tomato crop production, focusing on yield and quality optimization. A randomized complete block design (RCBD) was used to evaluate four irrigation intervals and three doses of Brassinolide and Chitosan each with one control treatment. The experiment was conducted over two consecutive years (2022-23) at the Agriculture Research Institute, Swat, Khyber Pakhtunkhwa. Four Irrigation Intervals (II) including (II1=daily, II2=3, II3=6, II4=9 days), along with varying concentrations of Brassinolide (3,6 and 9 $\mu\text{M L}^{-1}$) and Chitosan (100,200 and 300 mg L^{-1}), to determine their effects on tomato crop production and qualitative attributes. The study revealed significant variations in tomato plant parameters in response to different irrigation intervals and brassinolide (BL) + chitosan (CH) applications. 3-day irrigation intervals with BL 6 $\mu\text{M L}^{-1}$ + CH 100 mg L^{-1} resulted in the maximum fruits per plant (64.66), and yield (58.42 ton.ha^{-1}). The 9-day irrigation interval generally exhibited the poorest performance, with the control treatment demonstrating minimum values for various parameters, including fruits per plant (13.5), yield (3.41 tons.hac^{-1}), membrane stability index (35.56%), and leaf relative water content (52.88%). Biochemical Attributes data showed that the maximum Fruit Firmness (4.24 kg.cm^{-2}), Ascorbic Acid (21.82 mg.100g^{-1}), Titratable Acidity (0.65%), TSS (4.120Brix), and Reducing Sugars (3.36%) were recorded at six-day intervals. In contrast, the minimum was recorded at nine-day intervals. The foliar application, Maximum Fruit Firmness (4.39 kg.cm^{-2}), Ascorbic Acid (22.15 mg.100g^{-1}), Titratable Acidity (0.72%), and Reducing Sugars (3.12%) were recorded at Chitosan at 100 mg.L^{-1} While minimum Fruit pH (4.14) and maximum TSS (4.09) were recorded at combined foliar application of BL6+CH100.

INTRODUCTION

Tomato production in Pakistan has undergone significant evolution in recent years. The cultivated area expanded from 55,258 hectares to 69,498 hectares between 2018 and 2021, resulting in an increase in output from 561,293 tons to 694,204 tons (MNFSR, 2022). However, national production remains below the global average at 10 tons.ha^{-1} compared to 38 tons.hac^{-1} globally, indicating a disparity of approximately 286% (Younas et al., 2024). Tomatoes are crucial to Pakistan's agriculture, contributing over 4.2 million tons annually. Despite this, Pakistan's tomato exports generate only 28% of the global average export price, constituting less than 1% of its output, compared to a global average export-production ratio of 4.7% (MNFSR, 2022).

The tomato production in Pakistan is suffering from several problems which hinder its growth and sustainability. Increasing temperature and irregular rainfall brought about by climate change worsen crop yields and quality (Ali et al., 2021; Rehman et al., 2021).

Despite possessing high yield potential, access to effective irrigation infrastructure remains limited, thereby constraining the utilization of water resources. (Kirby et al., 2017; Raza et al., 2022). The need for irrigation in cash crops like tomatoes shows that disruption in water supply has a direct impact on production (Hassan et al., 2022). Inadequate water supply induces water stress, low RWC, poor plant growth and low yield and quality (Albasha et al., 2016). In tomatoes, photosynthetic efficiency is reduced and susceptibility to physiological disorders is increased when subjected to deficit irrigation, owing to lower relative water content (RWC) (Mendonça et al., 2020). This study aims to investigate the combined effects of Brassinolide and Chitosan on tomato yield and quality under different irrigation intervals, hypothesizing that optimized combinations of these treatments can significantly enhance crop performance.

The post-harvest quality of tomato (*Solanum lycopersicum*) is greatly affected by irregular water supply and intervals of irrigation practices. Inconsistent watering



induces physiological stress on fruit in terms of fruit firmness, color and nutritional content (Samui *et al.* 2020; Zhao *et al.*, 2019). Inadequate irrigation impedes soluble solids and sugar accumulation, vital for flavour and quality (Shao *et al.*, 2014; Zhao *et al.*, 2019). Waterlogging is caused by excessive irrigation and root health and nutrient uptake deterioration (Zhao *et al.*, 2019; Lovelli *et al.*, 2017). Consequently, irrigation interval and post-harvest quality are linked, as a result, knowledge of this relationship is essential for the development of sustainable and profitable water management strategies in water-scarce areas (Lovelli *et al.*, 2017; Boiteau & Pingali, 2022).

For instance, given the challenges of Irregular irrigation intervals which may cause water stress and over irrigation create water logging, the need for sustainable agricultural practices, this study explores Brassinolide and Chitosan, two biostimulants with the potential to improve plant resilience to stress and enhance yield and quality.

The natural plant growth regulator from the class of brassinosteroids, called brassinolide, may be the future of sustainable agriculture. This compound offers a choice other than the use of synthetic chemicals in that it enhances plant growth and resistance to environmental stress. For an application that should be running in parallel with the increasing desire for eco-friendly agricultural products and for consumers who are willing to pay extra for environmentally approved goods (Al-Turki *et al.*, 2023; Bano *et al.*, 2022). Brassinolide has been demonstrated to confer benefits by promoting cell elongation and division, which are crucial for biomass accumulation and leaf area growth, ultimately contributing to fruit development (Hu *et al.*, 2017). Brassinolide treatment has been shown to increase the relative water content in plant tissue so that water shortage effects are reduced (Li & Feng, 2011).

Chitosan is a biopolymer from chitin, a sugar polymer frequently found in the exoskeletons of arthropods and fungi, and it is a versatile agent for tomato (*Solanum lycopersicum*) growing, improving plant growth, yield and production via the enhancement of nutrient management and stimulating the physiological responses (Sharif *et al.* 2018). Chitosan proved to be a potential substance to enhance water use efficiency, relative water content and chlorophyll levels of tomato plants (*Solanum lycopersicum*) subjected to inconsistent water supply. Chitosan application improves physiological responses thereby increasing water retention and turgor pressure, both very important to growth in the water stress conditions (Hernández Hernández *et al.*, 2018; Attia *et al.*, 2021). Chitosan has also been studied to significantly enhance photosynthetic efficiency and plant health as this can increase chlorophyll a and b content of plants (Hernández Hernández *et al.*, 2018)

Brassinolide application changes physiological processes that cause retention of quality attributes such as firmness, colour and nutrient levels (Zhu *et al.*, 2015). It helps tomatoes accumulate lycopene, and improves the nutritional profile and colour (Zhu *et al.*, 2015). It inhibits the ethylene effect and increases shelf life of produce (Changjun *et al.*, 2021). This natural biopolymer also improves harvested produce firmness, color retention and nutritional content (Zhu *et al.*, 2015; Petriccione *et al.*, 2015). Two critical factors in protecting product freshness

and quality during storage are the respiration rate and moisture loss, both of which chitosan coating mitigates (Meena *et al.*, 2020; Oke *et al.*, 2020). For instance, it is applicable for perishable produce such as tomatoes that is vulnerable to post-harvest diseases leading to economic losses. In addition to that, chitosan is organic and biodegradable and adheres to the consumers' wish to consume eco-friendly so that it can be employed as a substitute for synthetic preservatives (Zakir *et al.*, 2022; Salman *et al.*, 2021)

Previous studies have indicated that Brassinolide concentrations between 0.5 to 15 $\mu\text{M L}^{-1}$ and Chitosan concentrations from 75 to 300 mg L^{-1} optimize plant growth and yield in various vegetable crops under different stress conditions (Ali *et al.*, 2019). Similarly, different irrigation intervals have been found to significantly affect tomato plant performance (Hassnain *et al.*, 2020)

This article investigates the combined effects of brassinolide and chitosan as biostimulants on tomato production and quality attributes under varying irrigation intervals. It examines their impact on, yield, and plant resilience by analyzing physiological and biochemical responses. The study aims to optimize application methods, concentrations, and timing to enhance production and quality attributes. Ultimately, this study seeks to develop sustainable, eco-friendly practices for resilient tomato production in the face of climate change and water scarcity challenges.

METHODOLOGY

Experimental site description

The study was conducted at the Agriculture Research Institute Swat in Khyber Pakhtunkhwa province. The climate was sub-humid, with an annual mean temperature of 19.3°C. Physical and chemical analyses of the soil were performed before plant transplantation within the 0-20 cm soil layer. The soil was classified as silt loam.

Experimental Design and Treatment Details

A randomized complete block design (RCBD) was employed to assess four irrigation intervals and three doses each of Brassinolide and Chitosan. The experiment evaluated daily irrigation (II1), irrigation every third day (II2), every sixth day (II3), and every ninth day (II4) as irrigation intervals. Brassinolide concentrations included a control (0 μML^{-1}), 3 μML^{-1} (BL3), 6 μML^{-1} (BL6), and 9 μML^{-1} (BL9), while Chitosan concentrations were 0 mgL^{-1} (CH0), 100 mgL^{-1} (CH100), 200 mgL^{-1} (CH200) and 300 mg L^{-1} (CH300). Treatment combination of Brassinolide and Chitosan were Brassinolide (BL), Chitosan (CH) = 0, BL1=3, BL2=6, BL3=9, CH1=100, CH2=200, CH3=300, BL1+CH1=3+100, BL1+ CH2=3+200, BL1+CH3=3+300, BL2+CH1=6+100, BL2+CH2=6+200, BL2+CH3=6+ 300, BL3+CH1=9+100, BL3+CH2=9+200, and BL3+CH3=9+300. To ensure reproducibility and reliability of results, the experiment was conducted over two consecutive years.

Nursery Raising and Transplantation

Seeds of the Rio-Grande variety were sown in April, and transplantation was subsequently performed in the first week of May in both years (2022-23). Healthy and vigorous

seedlings were selected for transplantation. The transplantation of seedlings was conducted in the evening to mitigate direct heat exposure and facilitate the adaptation of seedlings to transplantation stress. A lath house structure was constructed using galvanized iron (G.I.) pipes to protect the experimental plot from rainwater.

Irrigation Intervals Management

The irrigation water was determined using evapotranspiration and Time domain reflectometry (TDR). During the initial 20 days following transplantation, standard irrigation practices were employed to facilitate plant establishment. Different irrigation intervals were implemented after 20 days of the post-transplantation of tomato seedlings. The number of irrigations and the quantity of water applied during each irrigation interval were measured. Four irrigation intervals were implemented, with varying numbers of irrigation events and applied water quantities.

Studied Parameters

The number of fruits per plant was determined as the average of the number of fruits per plant was counted from the tagged plant at each picking until the last harvest, fruits considering all plants from the sampling plot. The average fruit weight was calculated by measuring the weight of randomly selected fruits from each plant and then dividing this total weight by the number of fruits harvested from that same plant. Very small, misshapen and cracked fruits were considered unmarketable. The yield in tons per hectare was calculated by multiplying the average fruit yield per plant (kg) from each treatment by the average number of plants per hectare, then dividing by 1000 to obtain the yield in tons per hectare.

$$\text{Average Number of Plants ha}^{-1} \times \text{Average Fruit Yield Plant}^{-1}(\text{kg}) / 1000$$

Membrane Stability Index %

Ten leaf discs of 200 mg (10mm in diameter) were picked from the fifth leaf from the apex and placed in tubes containing 10 ml of double distilled water in two sets. One set was subjected to 40°C for 30 min in a water bath, and its electrical conductivity (EC1) was determined after the incubation period using an electrical conductivity meter. The second set of tubes was heated in a temperature-controlled water bath at 100°C for 15 min, after which electrical conductivity (EC2) was measured. The membrane stability index was calculated as a percentage (Fawzy *et al.*, 2019).

$$\text{Membrane Stability Index (MSI)}(\%) = 1 - \frac{(EC1)}{(EC2)} \times 100$$

Leaf Relative Water Content (LRWC %)

To determine the Leaf Relative Water Content (LRWC), 20 leaf disc samples were obtained using a cork borer, taking discs with a diameter of 10 mm from the fifth leaf from the apex. These discs were placed in a pre-weighed Petri dish to record the fresh weight (F. Wt.). The discs were subsequently immersed in distilled water in a sealed Petri dish for 24 hours to achieve full turgidity. After this period, the discs were carefully weighed again after removing excess water to determine the turgid weight (T. Wt.). Next, the leaf discs were placed in a preheated oven at 70°C until

a constant weight was reached, which took approximately 48 hours. They were weighed again to obtain the dry weight (D. Wt.). The LRWC percentage was calculated using the formula proposed by Fawzy *et al.* (2019).

$$\text{LRWC \%} = [(FW-DW)/(TW-DW)] \times 100$$

Quality Attributes

The Fruits of uniform size and color, free from mechanical injury, were selected for quality attributes determination. Fruit from each treatment were harvested and brings to the laboratory. For each sample, four fruits were selected of similar size and maturity. Fruit firmness was assessed using a Penetrometer (FTFT011, Italy) with a 4 mm probe. Five fruits were randomly selected from each treatment plot. Uniform pressure was maintained, and the mean firmness was recorded in kg.cm⁻², ensuring a systematic and consistent procedure across all samples. A portable refractometer was used to determine the total soluble solid content of the tomato juice, expressed in degrees Brix (°Brix). The pH of fruit juice was determined using an electronic pH meter. Before analysis, the pH meter was calibrated. The procedure for determining titratable acidity (TTA) in tomato fruits followed the AOAC 2006 method, which involved a precise titration process. (g.100g⁻¹ FW) was measured by titration using 0.1 N NaOH against 4:1 dilution of tomato extract with water. All sample were performed thrice for each sample to ensure accuracy. Reducing sugars were determined using lane and Eynon titration method described in AOAC 2000. The method was based on the reduction of Fehling's solution in the presence of heat and methylene blue indicator.

Statistical Analysis

Data analysis was performed using STATISTIX 8.1 software and statistically analyzed using analysis of variance (ANOVA). The least significant difference test (LSD) at (p<0.01) was applied to identify significant difference among means (steel *et al.*, 1997).

RESULTS AND DISCUSSION

Number of Fruits Plant⁻¹ and yield ton Hectare⁻¹

The number of fruits per plant and yield ton Hectare⁻¹ in tomatoes was significantly influenced by varying irrigation intervals and foliar application of Brassinolide (BL) and Chitosan (CH). The interaction between irrigation intervals (II) and Brassinolide-Chitosan BL+CH was also significant. However, data regarding years and the interactions between (II×Y), (Y×BL + CH), and (Y × II × BL + CH) were not significant (Table 1).

The results revealed that tomato plants irrigated at three-day intervals produced the maximum number of fruits per plant (55.51) and yield (45.19 tons.ha⁻¹), followed by plants irrigated daily (49.02) and yield (37.10 tons.ha⁻¹). The minimum number of fruits per plant (20.07) and yield (7.36 tons.ha⁻¹) was observed in plants irrigated at nine-day intervals (Table 1).

Regarding the foliar application of BL and CH, a concentration of 6 µmL⁻¹+CH100 mgL⁻¹ yielded the highest number of fruits per plant (49.63) (37.81 tons. ha⁻¹), followed by the foliar application of BL6 µmL⁻¹, which produced 46.96 fruits per plant and yield (34.96 tons. ha⁻¹). Chitosan application at 100 mgL⁻¹ resulted in 43.71 fruits per plant and a yield (30.82 tons. ha⁻¹). The lowest

number of fruits per plant was observed in the control treatment (30.71) and yield (3.48 tons.ha⁻¹). Table (1)

The interaction between irrigation intervals and BL + CH concentrations demonstrated that the maximum number of fruits per plant (64.66) and yield (58.42 tons.ha⁻¹) was achieved with three-day irrigation intervals combined with BL (6 µmL⁻¹) + CH (100 mgL⁻¹). The minimum number of fruits per plant (13.5) and yield (3.48 tons.ha⁻¹) was recorded at nine-day irrigation intervals at controlled applied plants. (Fig. 1 and 2).

Leaf Relative Water Content (%) and membrane stability index

Analysis of variance (ANOVA) revealed a significant effect of irrigation intervals, foliar application of Brassinolide + Chitosan, and their interaction (II × BL + CH) on leaf relative water content and membrane stability index of tomato ($p \leq 0.01$). However, no significant effects were observed between the interaction of Y × II, Y × BL + CH, and Y × II × BL + CH (Table 1).

Irrigation intervals significantly influenced the leaf's relative water content. The highest leaf-relative water content (83.30%) and membrane stability index (61.46%) was observed in plants irrigated daily, followed by those irrigated every three days (77.55%), (58.26%). The lowest leaf relative water content (60.11%) and membrane stability index (43.55%) was recorded in plants irrigated at 9-day intervals (Table 1).

Table 1

Number of Fruits Plant-1, Yield ton Hectare-1, Leaf Relative Water Content (%) and Membrane stability index (%) of tomato as affected by irrigation intervals, Brassinolides and chitosan treatment

Irrigation Intervals (Days)	Number of Fruits Plant ⁻¹	Fruit Yield ton hectare ⁻¹	Leaf Relative Water Content (LRWC)	Membrane Stability Index %
1	49.02B	37.10B	83.30A	61.46A
3	55.51A	45.19A	77.55B	58.26B
6	40.36C	24.53C	70.32C	50.88C
9	20.07D	7.36D	60.11D	43.55D
LSD ($P \leq 0.01$)	0.37	0.30	0.28	0.43
Brassinolide (µmL ⁻¹) + Chitosan (mg L ⁻¹)				
0	30.71J	17.25L	67.30I	48.47I
3	40.42E	27.92G	72.86E	53.29E
6	46.96B	34.96B	75.89B	57.00A
9	43.50C	32.39D	73.30E	54.20D
100	43.71C	30.82EF	74.50D	56.36AB
200	39.92EF	27.36GH	71.99F	52.81EF
300	38.46G	24.94I	71.04G	51.89G
3+100	46.42B	33.76C	75.72BC	55.81BC
3+200	43.25CD	31.16E	74.06D	55.41C
3+300	37.67H	24.03J	70.90G	50.37H
6+100	49.63A	37.81A	77.30A	57.16A
6+200	42.67D	30.76EF	73.11E	54.45D
6+300	38.08GH	24.64I	70.62G	50.67H
9+100	43.21CD	30.53F	75.32C	55.86BC
9+200	39.54F	27.06H	71.61F	52.33FG
9+300	35.75I	21.34K	69.63H	50.49H
LSD ($P \leq 0.01$)	0.74	0.255	0.56	0.87
Year				
2022	41.10	28.52	73.20	53.47
2023	41.39	28.57	72.45	53.60
LSD ($P \leq 0.01$)	NS	NS	NS	NS
Interactions				
I × T	Fig 1	Fig 2	Fig 3	Fig 4
Levels of Significance	***	***	***	***
Y × I	---	---	---	---
Levels of Significance	NS	NS	NS	NS
Y × T	---	---	---	---
Levels of Significance	NS	NS	NS	NS
Y × I × T	---	---	---	---
LSD ($P \leq 0.01$)	NS	NS	NS	NS

The mean values for BL +CH treatments indicated that the combined application of BL 6 µmL⁻¹ + CH100 mgL⁻¹ recorded the highest leaf relative water content (77.30%) and membrane stability index (57.16%), followed by Brassinolide alone (75.89%) (57.0%). Chitosan at 100 mgL⁻¹ recorded leaf relative water content (74.50%), (56.36%). The lowest leaf-relative water content (67.30%) and membrane stability index (48.47%) was observed in controlled plants (Table 1).

The interaction between irrigation intervals and Brassinolide + Chitosan treatments demonstrated that the maximum leaf relative water content (84.94%) and membrane stability index (63.18%) was achieved with daily irrigation and the BL 6µmL⁻¹ treatment. This was closely followed by daily irrigation with BL6 µmL⁻¹ + CH100 mgL⁻¹ (84.58%), while in case of membrane stability index 63.03% was recorded at BL3 µmL⁻¹+CH200 mgL⁻¹. Conversely, the minimum leaf relative water content (52.72%), (35.56%) was recorded at 9-day irrigation intervals in controlled treated plants (Fig. 3 and 4).

A clear trend was observed in leaf relative water content and membrane stability index, which decreased as irrigation intervals increased from daily to 9-day intervals. However, no consistent trend was noted with increasing concentrations of BL (3 µmL⁻¹ to 9 µmL⁻¹) or CH (100 mgL⁻¹ to 300 mgL⁻¹).

Means followed by similar columns are not significantly different from each other α 0.05. NS = non-significant. * = Sig at 5% and **, *** = sig at 1% level of probability. I=Irrigation Intervals, T= treatments (Brassinolide - Chitosan) and Y= Year, μML^{-1} = Micromole per litre, mg L^{-1} = milligram per litre.

Figure 1

The Interaction between Irrigation Intervals and Brassinolide-Chitosan on Number of Fruits Plant⁻¹.

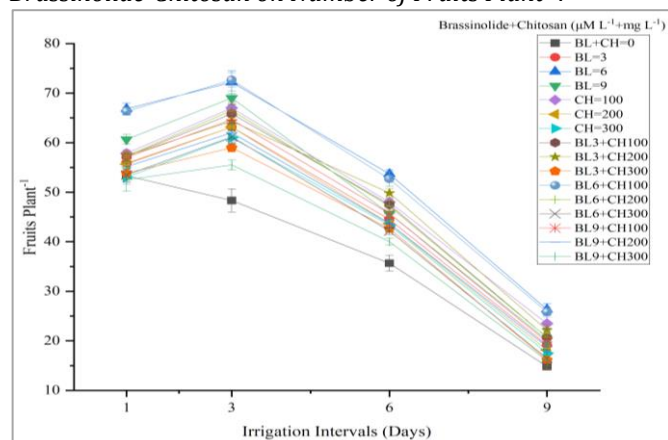


Figure 2

The Interaction between Irrigation Intervals and Brassinolide-Chitosan on Yield ton Hectare⁻¹.

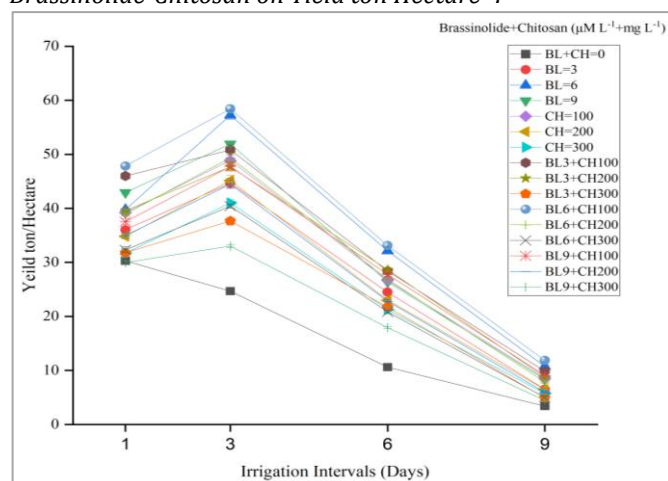


Figure 3

The Interaction between Irrigation Intervals and Brassinolide-Chitosan on Leaf Relative Water Content %.

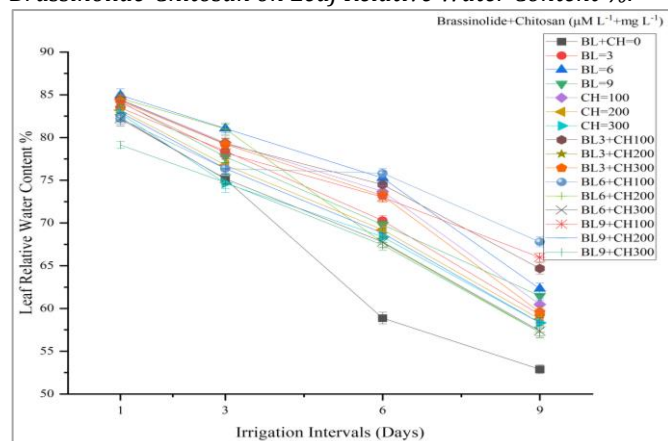
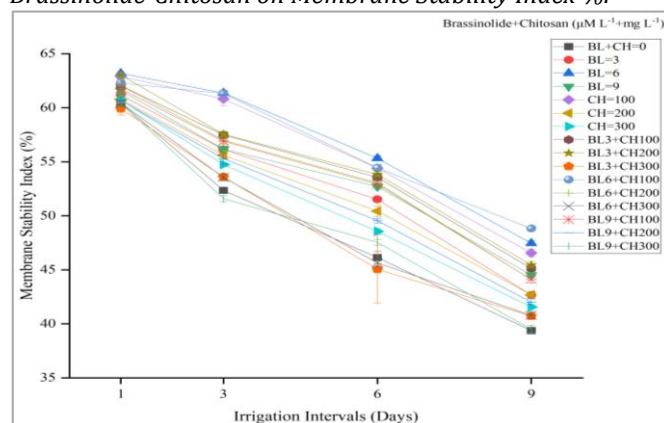


Figure 4

The Interaction between Irrigation Intervals and Brassinolide-Chitosan on Membrane Stability Index %.



Irrigation intervals had significant relationships with tomato yield components such as fruit per plant, yield per hectare. Long intervals lead to the reduction of soil moisture, hinder nutrient and water uptake, limit growth, and decrease fruit production (Ayas, 2019). Muroyiwa (2023) further added that adequate water supply promotes production as it facilitates nutrient absorption and physiological functions that are critical for the development of fruits. Irrigation is essential for plants to maintain their metabolic processes and for higher fruit set, size, and yield. Lovelli *et al.* (2017) reported that interrupting irrigation during critical growth periods lowered the marketable yield. A long-term deficit irrigation may also significantly decrease the yield (Mukherjee *et al.*, 2023).

Brassinolide plays a role in hormone balance, particularly of the auxins and the gibberellins, which are both important for flower and fruit development. Plants applied with Brassinolide have increased fruit weights and yields. According to studies, brassinolide-treated plants grow significantly higher yields than control plants (Ghosh *et al.*, 2022). Yield and quality in different crops are enhanced under stress conditions while applying brassinolide (Swain *et al.*, 2023). Foliar Application of brassinosteroids also improved the yield parameters and fruit yield of the tomato, as BRs are known to delay the senescence process, that's why more number of flowers per cluster will be retained which ultimately enhances number of fruits and yield of tomato. (Jangid & Dwivedi, 2017; wang, 2019). In tomato plants which were applied homobrassinolide it is also been reported improved fruit set by the regulation of source to sink relation due to maximum photosynthetic rate (Sridhara *et al.*, 2021).

Chitosan improves tomato yield by improving nutrient availability and uptake, especially nitrogen, which causes better vegetative growth and fruit yield (Ullah *et al.*, 2020). It was found that chitosan increases stomatal conductance and net photosynthetic activity, which increases plants' tolerance to abiotic stresses such as water scarcity and maintains biomass production under water-limited conditions (Farouk & Amany, 2012). Chitosan affects hormonal equilibrium by enhancing the growth-regulating hormones that are essential in flower and fruit development, which leads to higher flowers and better growth indicators, as mentioned by Amerany *et al.* (2020)

Leaf relative water content is an important physiological indicator of the plant, particularly under different irrigation intervals. The effects of irrigation intervals on RWC have been studied, demonstrating that water availability plays a significant role in plant health and yield.

Water stress due to inadequate water supply decreases LRWC in tomato plants. Drought stress affects chlorophyll content, overall growth and decreases the LRWC by 20% compared to those that received sufficient irrigation (Turan *et al.*, 2023). Similar findings of Yilmaz also showed that infrequent irrigation adversely affects chlorophyll content, which is related with photosynthetic activity, and alternatively affects leaf relative water content (Yilmaz and Korkmaz, 2021). According to the study, the foliar application of chitosan improves Relative Water Content (RWC) by increasing stomatal conductance and chlorophyll levels, which are necessary for photosynthesis and water retention (Demehin *et al.*, 2024). This substance helps plants adapt to drought stress by improving RWC and reducing lipid peroxidation. Besides, chitosan also increases nutrient absorption and water use efficiency, resulting in better growth and yield under water-limited situations (Mohammed *et al.*, 2024)

According to a study, brassinosteroid application can increase stomatal conductance, thus increasing gas exchange and decreasing water loss through transpiration (Ji, 2023). The relative water content (RWC) is also indirectly boosted by brassinolide through the regulation of antioxidant defense mechanisms and the promotion of the production of protective compounds. (Ji, 2023; Naservafaei *et al.*, 2021). Applying brassinolide in tomato plants increases photosynthetic capacity and decreases malondialdehyde (MDA) accumulation, maintaining plant health and water retention (Zhang *et al.*, 2021).

Quality attributes

The statistical analysis revealed that irrigation intervals and foliar application of Brassinolide and Chitosan significantly influenced the Fruit Firmness, Ascorbic acid, titratable acidity, total soluble solids, fruit juice pH, and reducing sugars of tomato. However, no significant effects were observed for the interaction of (II $\times$ BL + CH), (Y $\times$ II), (Y $\times$ BL CH), and Y $\times$ II $\times$ BL CH (Tables 2 and 3).

Statistical analysis demonstrated that plants irrigated at six-day intervals exhibited the maximum fruit firmness (4.24 kg.cm⁻²), Ascorbic Acid (21.82 mg.100g⁻¹), Titratable Acidity (0.65%), TSS (4.12 °Brix) and reducing sugars (3.36%) followed by those irrigated at three-day intervals (4.18 kg.cm⁻²), (21.39) (0.62%), (3.89°Brix) and (3.16%). The plants subjected to nine-day irrigation had the lowest Fruit Firmness (3.96 kg.cm⁻²), Ascorbic acid (21.39 mg.100g⁻¹), titratable acidity (0.57%), TSS (3.61°Brix) and reducing sugars (2.68%) (Tables 2 and 3).

Foliar application of various concentrations of Brassinolide and Chitosan resulted significant effect on tomato quality attributes. The maximum Fruit Firmness (4.39 kg.cm⁻²), Ascorbic Acid (22.15 mg.100g⁻¹), titratable acidity (0.72%) and reducing sugars (3.18%) was recorded with the application of CH100 mgL⁻¹, followed by the combined application of Brassinolide and Chitosan 6 μ ML⁻¹+CH100 mgL⁻¹ Fruit firmness (4.38 kg.cm⁻²),

Ascorbic acid (22.13 mg.100g⁻¹), titratable acidity (0.71%) and Brassinolide alone at 6 μ ML⁻¹ (4.35 kg.cm⁻²), (21.90 mg.100g⁻¹), (0.67%). While the maximum TSS (4.09 °Brix) and minimum Fruit juice pH (4.14) were recorded at the combined foliar application of Brassinolide and Chitosan (100+6). The control treatment exhibited the lowest fruit firmness (3.93 kg.cm⁻²), ascorbic acid (20.84 mg.100g⁻¹), titratable acidity (0.54%), TSS (3.64°Brix), and reducing sugars (2.96%). (Tables 2 and 3).

Table 2

Fruit Firmness (kg.cm⁻²), Ascorbic Acid (mg/100g), and Titratable Acidity (%) of tomato as affected by irrigation intervals, Brassinolides and chitosan treatment

Irrigation Intervals (Days)	Fruit Firmness (kg.cm ⁻²)	Ascorbic Acid (mg/100g)	Titratable Acidity (%)
1	4.11 B	21.1558 B	0.606 BC
3	4.18 AB	21.3925 B	0.629 AB
6	4.24 A	21.8239 A	0.657 A
9	3.96 C	20.7272 C	0.570 C
LSD (p $\leq$ 0.01)	0.095	0.4032	0.0366
Brassinolide (μ ML ⁻¹) + Chitosan (mgL ⁻¹)			
0	3.93 E	20.84 D	0.54 FG
3	4.07 CDE	21.07 CD	0.59 EFG
6	4.35 A	21.90 AB	0.67 ABC
9	4.23 ABC	21.15 BCD	0.68 ABC
100	4.39 A	22.15 A	0.72 A
200	3.99 E	21.05 CD	0.59 EFG
300	4.00 E	21.08 CD	0.57 EFG
3+100	4.27 AB	21.68 ABC	0.54 G
3+200	4.04 CDE	20.85 D	0.65 BCD
3+300	4.07 CDE	20.84 D	0.63 CDE
6+100	4.38 A	22.13 A	0.71 A
6+200	4.00 E	21.17 BCD	0.54 FG
6+300	4.02 DE	21.02 CD	0.61 DEF
9+100	4.21 BCD	21.41 ABCD	0.69 AB
9+200	4.08 CDE	21.19 BCD	0.57 EFG
9+300	3.98 E	20.88 CD	0.56 EFG
LSD (p $\leq$ 0.01)	1.195	0.8064	0.732
Years			
2022	4.13	21.30	0.54
2023	4.15	21.36	0.54
LSD (p $\leq$ 0.01)	NS	NS	NS
Interactions			
I $\times$ T	NS	NS	NS
Y $\times$ I	NS	NS	NS
Y $\times$ T	NS	NS	NS
Y $\times$ I $\times$ T	NS	NS	NS

Means followed by similar columns are not significantly different from each other at P $\leq$ 0.01. NS = non-significant. * = Sig at 5% and **, *** = Sig at 1% level of probability. I=Irrigation Intervals, T= treatments (Brassinolide - Chitosan), Y= Year, LSD = Least significant difference.

Table 3

Fruit Firmness (kg.cm⁻²), Ascorbic Acid (mg/100g), and Titratable Acidity (%) of tomato as affected by irrigation intervals, Brassinolides and chitosan treatment

Irrigation Intervals (Days)	TSS (°Brix)	Fruit juice pH	Reducing Sugars (%)
1	3.72 C	4.19	2.78 C
3	3.89 B	4.18	3.16 B
6	4.12 A	4.18	3.36 A
9	3.61 D	4.20	2.68 D
LSD (p $\leq$ 0.01)	0.1014	NS	0.050
Brassinolide (μ ML ⁻¹) + Chitosan (mgL ⁻¹)			
0	3.64 F	4.22 AB	2.967 DE

3	3.74 EF	4.20 AB	2.958 DE
6	4.07 A	4.21 AB	3.096 AB
9	3.89 CDE	4.23 A	3.015 CDE
100	4.05 AB	4.18 BC	3.123 A
200	3.79 DEF	4.21 AB	2.968 DE
300	3.71 EF	4.17 BC	2.939 E
3+100	3.95 ABC	4.19 AB	3.029 CDE
3+200	3.86 CDE	4.21 AB	2.946 E
3+300	3.71 EF	4.20 AB	2.961 DE
6+100	4.09 A	4.14 C	3.074 ABC
6+200	3.80 DEF	4.18 ABC	2.999 CDE
6+300	3.70 EF	4.18 ABC	2.973 DE
9+100	3.94 BCD	4.14 C	3.058 BCD
9+200	3.78 DEF	4.20 AB	2.974 DE
9+300	3.65 F	4.21 AB	2.939 E
LSD ($p \leq 0.01$)	0.20	0.05	0.10
Years			
2022	3.85	4.2	2.99
2023	3.84	4.2	2.98
LSD ($p \leq 0.01$)	NS	NS	NS
Interactions			
I x T	NS	NS	NS
Y x I	NS	NS	NS
Y x T	NS	NS	NS
Y x I x T	NS	NS	NS

Means followed by similar columns are not significantly different from each other at $P \leq 0.01$. NS = non-significant. * = Sig at 5% and **, *** = Sig at 1% level of probability. I=Irrigation Intervals, T= treatments (Brassinolide – Chitosan), Y= Year, LSD = Least significant difference.

Irrigation intervals affects the quality parameters of tomato fruits i.e. fruit pH, firmness, total soluble solids (TSS), titratable acidity, reducing sugar. For fruit, pH, which is important, was quite stable among irrigation intervals. Deficit irrigation had a positive response to fruit firmness. According to Lu *et al.* (2021), firmness was shown to increase by 12.09% under DI conditions. The main reason for structural integrity is due to this water being reduced inside the fruit, which means the concentration of cell wall components is higher. Improving firmness is important for the fruit quality, postharvest handling and shelf life, and reduction in weight loss. The effect of reduced irrigation was consistently positive on total soluble solids (TSS), an important attribute and taste determinant of fruit quality. This increase was mostly due to a concentration effect brought about by less water in the fruit resulting in a higher concentration of soluble solids. As titratable acidity also increases under reduced irrigation regimes, the probable cause of the increase in organic acids in the fruit is due to stress due to water limitation (Lu *et al.*, 2021; Chen *et al.*, 2013). Most of the sweetness of tomatoes depends on the reduction of sugars, glucose, and fructose. Deficit irrigation has further increased the sugar content (Lu *et al.*, 2015; Lu *et al.*, 2021). This may be part of the increase due to the increased conversion of starch to sugars under water stress.

brassinolide affects the TSS and ascorbic acid of tomatoes, and homobrassinolide enhances TSS, which can be interpreted as higher sugar content and sweetness (Sridhara *et al.*, 2021). Brassinolide may indirectly affect the reducing and non-reducing sugar content by increasing the total amount of sugar. Fruit firmness is improved by HBR (Sridhara *et al.*, 2021) with the HBR treatment of 0.12 g.ha^{-1} , giving 4.11 kg cm^{-2} firmness,

which is higher than the control. BRs potentially do this because they increase cell wall metabolism and structural integrity. Ethylene biosynthesis is important for fruit ripening, and BRs promote ethylene biosynthesis. Sugar accumulation and flavor development in tomatoes can be increased with increased ethylene levels (Hu *et al.*, 2020). Carotenoid biosynthetic gene regulation by BRs leads to better fruit color and nutritional quality and indirectly influences fruit-perceived sweetness and fruit quality (Hu *et al.*, 2020). BR application use enhances chlorophyll content and photosynthetic efficiency and, therefore, sugar production, which may be manifested in higher sugar levels in fruits (Júnior *et al.*, 2022; Maia *et al.*, 2018).

Zhang *et al.* (2017) reported that chitosan treatment did not affect pH significantly, but other quality attributes were improved, such as firmness, total phenolics, and flavonoids. However, Meena *et al.*, (2020) reported that chitosan treatment helped to keep fruit firm during storage, which is essential for improving shelf life and reducing post-harvest losses. It has been shown by several studies that the chitosan treatment has the ability to preserve TSS and titratable acidity levels, which are vital for the taste and, in general, the quality of the fruit (Shehata *et al.*, 2021; Meena *et al.*, 2020). This is probably because the chitosan reduced the respiration rate and delayed the fruit ripening process, leaving the fruit's biochemical composition unaltered. Chitosan treatments that maintain the same or higher sugar levels or titratable acidity, causing an increase in the sugar-acid ratio and improving the flavor of the tomatoes, are good for tomatoes (Shehata *et al.*, 2021; Meena *et al.*, 2020). Several factors contribute to the beneficial effect of chitosan on tomato fruit quality. According to Amerany *et al.* (2022) and Attia *et al.* (2021), it is reported that chitosan is able to enhance plant defense response, which decreases microbial infection and physical damage to the fruit.

CONCLUSION

Three-day irrigation intervals were most effective for maximizing yields performance of tomato both number of fruits per plant and the yield tons per hectare, while daily irrigation of plants has recorded maximum leaf relative water content and membrane stability index. Nine-day irrigation intervals generally led to suboptimal plant performance, including increased stress responses such as decreased leaf relative water content and reduced membrane stability index, which ultimately affected the yield performance of tomato. The combined application of Brassinolide ($6 \mu\text{M L}^{-1}$) and Chitosan (100 mg L^{-1}) significantly improved tomato yield, and physiological parameters compared to the concentrations applied alone, other combinations and also control. Biochemical attributes were improved when plants were irrigation on three- and six-day intervals with BL6, CH100, and their combination. However, the best result was obtained with Chitosan applied at 100 mg L^{-1} at six-day intervals. Three-day irrigation intervals with combined application of brassinolide and chitosan at concentrations of ($6 \mu\text{M L}^{-1} + 100 \text{ mg L}^{-1}$) are recommended for maximum yield of tomato and chitosan at 100 mg L^{-1} , and combined

application of brassinolide and chitosan (6 μML^{-1} +100mgL⁻¹) with six-day irrigation intervals and three-

day irrigation intervals is recommended for improvement of quality attributes of tomato.

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