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Comparative response of tomato seedlings to silicon and seaweed biostimulants under irrigation-frequency and post-emergence darkness stress

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Abstract

Tomato seedlings (*Solanum lycopersicum* L.) were evaluated in a 6 × 3 factorial design laid out in a randomized complete block design with three replications, testing seaweed extract (AgraAlgen) and silicon-based biostimulant (CibuSilMax [CibuSil]) under multiple stresses (three irrigation intervals [once, twice, and thrice daily] and three post-emergence darkness durations [0, 2, and 4 days]). The absence of an untreated control limits interpretation to comparisons of responses between the two biostimulants. Prolonged post-emergence darkness duration reduced several quality traits, while irrigation frequency had trait-specific effects. CibuSil enhanced stem elongation and branching, whereas AgraAlgen was more effective in increasing root development and foliar biomass under the combined stress of prolonged post-emergence darkness duration and variable irrigation intervals. These findings highlight the potential of biostimulants to improve seedling resilience to multiple abiotic stresses, including post-emergence darkness duration during germination and limited moisture availability. Overall, CibuSil improved the seedling height, seedling branching and the leaf area; while AgraAlgen improved root development and biomass under moderate stress. On the other hand, AgraAlgen predominantly improved biomass accumulation and root health, particularly under extended post-emergence darkness duration conditions compared to CibuSil. Consistent with ANOVA results, CibuSil may therefore be considered the more effective biostimulant under moderate irrigation frequencies, while AgraAlgen remains a beneficial option for enhancing below-ground growth under stress. Results from this study can serve as a pilot for future studies, suggesting that CibuSil may be more effective than AgraAlgen in mitigating irrigation stress; however, extended post-emergence darkness duration negatively impacts seedling health regardless of biostimulant use.

Key words: AgraAlgen, CibuSilMAX, germination, irrigation intervals, tomato seedlings

Introduction

A significant threat to agricultural productivity and food security is the environmental unpredictability, including erratic rainfall regimes with irregular onsets and inconsistent amounts of rainfall, rising air temperatures, and an increase in weather-related events like frosts, floods, droughts, and pests and crop diseases linked to climate change (Myeni and Moeletsi 2023). By 2030, it is predicted that these weather-related risks will become more frequent and significant, potentially pushing 122 million more people into extreme poverty (Jafino *et al.* 2020). Most horticultural crops, including tomato, are particularly vulnerable to erratic precipitation events at early growth stages (Jędrszczyk *et al.* 2016; Karienye *et al.* 2020). Water is essential for plants to grow successfully (Ors *et al.* 2021); and as a result, providing the optimal amount and quality of water in a timely manner is essential. Various studies have demonstrated that inefficient irrigation can significantly affect plant health.

For instance, Puértolas and Dodd (2022) demonstrated that different irrigation frequencies affect water status, gas exchange, foliar hormone profiles, and biomass in tomato and basil crops. In another study conducted by Barzegar *et al.* (2022), a loss of approximately 25% in the yield of tomatoes was recorded due to water stress, which reduced the growth parameters, including the number of leaves and the plant height. Pires *et al.* (2011) reported that tomato production in greenhouses depends primarily on the substrate, and the irrigation frequencies; in their study, it was evident that high irrigation frequency affected the production across the production cycle. Besides uneven irrigation frequencies, germinating seedlings often face low light stress (Gómez-Cabezas and España 2024), particularly in horticultural nurseries and off-season cultivation systems, triggering skotomorphogenesis, which is characterized by etiolation, inhibited leaf development, and altered growth in prolonged exposures (Casal 2013; Humplík *et al.* 2015).

To overcome the adverse effects of combined low-light stress and variations in irrigation intervals, treatments such as seaweed (*Ascophyllum nodosum*) extract-based biostimulants (Subramaniyan *et al.* 2023) and silicon (Ullah *et al.* 2016) have emerged as a promising agronomic strategy, enhancing crop resilience and facilitating recovery in multi-stress environmental conditions (Asif *et al.* 2023; Legodi *et al.* 2024). These fertilizer-free compounds enhance resilience and promote recovery from abiotic and biotic stressors (Rai *et al.* 2021). Biostimulants are well known as a mitigating strategy for promoting plant resilience under stress conditions (Khetsha *et al.* 2022). These novel products trigger molecular and physiological responses when applied to crops (Rouphael and Colla 2020); however, only a few studies have reported the recovery responses of combined- and multi-stress alleviation using biostimulants.

Therefore, the aim of this study was to determine the potential

effects of seaweed extract- and Si-based biostimulants on the germination and seedling growth of tomatoes (*Solanum lycopersicum* L.) subjected to variations in irrigation intervals and prolonged post-emergence darkness duration.

Materials and methods

Experimental site and crop management practices: The experiment was conducted in a greenhouse tunnel (12 m x 6 m), 72 m^2, located at the Central University of Technology, Free State (29°07'S, 26°12'E), South Africa. The experimental site is situated in a semi-arid region, at ca. 1390 m a.s.l. The greenhouse temperature was kept constant at 26°C, with a fan set to run if the temperature reached 30°C or higher. This experiment was conducted during the 2024 summer/autumn (March to April) growing season.

General plant material and crop management practices: Tomato hybrid STAR 9037 seeds (Stark Ayres, Bloemfontein, RSA) were sown in seedling pots filled with Bark Unlimited Organics substrate (Pretty Gardens, RSA), arranged in multipot trays (150 × 230 × 65 mm) with six seeds per pot, which served as the experimental unit. Each multipot tray had six cells/pots, with one seed per cell; with each treatment represented by a miltipot with six cells. Plants were monitored twice daily for pests; no predatory insect infestations were observed. Plants were

Experimental layout and treatment design: The study followed a 3 × 2 × 3 factorial design, scientifically representing three darkness durations, two biostimulants and three irrigation intervals. However, as illustrated in Table 3, the mean separation between the darkness durations and biostimulants was considered combined, thereby, converting the design into a 6 × 3 factorial design , where factor A comprised six post-emergence darkness duration (referred to as duration of post-emergence darkness duration exposure [Table 3]) × biostimulant combinations and factor B comprised three irrigation frequencies. The factorial design was arranged in a randomized complete block design with three replicates, illustrated in Table 3. The absence of an untreated control restricts interpretation to comparative responses between the two biostimulants.

Plants were irrigated at intervals once, twice, or three times daily at 08:00, 14:00, and 16:00 h. The total daily irrigation volume was calculated as:

$$Vd=N\times4$$

where *Vd* is the daily irrigation volume (mm $pot^{-1}.day^{-1}$), and *n* is the irrigation frequency per day (1, 2, or 3), resulting in approximately 4, 8, and 12 mm $pot^{-1}.day^{-1}$, respectively.. To achieve this, each plant was irrigated with manually using a RedRhino 2 L precision sprayer calibrated to 0.3 MPa for five minutes per irrigation, applying a nutrient solution (Table 1) formulated according to Combrink (2018) and adjusted to water analyses (Table 2), no leaching was observed during the experiment (Ngcobo *et al.* 2024; Liu *et al.* 2019).

The seedlings received foliar applications of seaweed extract-based biostimulant (AgraAlgen) and Si (CibuSilMAX, hereinafter CibuSil) (Supplier: AgraForum) at a weekly interval. The treatment schedule consisted of three applications, starting on March 30th, 2024 (7 days post-emergence), with subsequent applications on April 6th and 13th, 2024. The biostimulant treatments were applied with a 2 L pressure sprayer calibrated to 0.3 MPa, at a dosage of 30 mL per tomato seedling. The sprayer

Table 1. Standard Steiner nutrient solution used to grow tomatoes (Combrink 2018)

EC mS	Ions (mmol L^{-1})							
cm^{-1}	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	HCO_3^-
2.19	1.00	7.00	8.50	3.50	12.50	1.00	6.38	0.40

Table 2. Mineral water analysis for the Central University of Technology, Free State experimental site in all experiments (Testit-labs, RSA)

Minerals, pH, conductivity and alkalinity	Quantity	
	2022/23	2023/24
Calcium (mg L^{-1})	42.37	33.95
Nitrate (mg L^{-1})	0.38	0.78
Potassium (mg L^{-1})	7.67	4.66
Sulphate (mg L^{-1})	27.48	18.96
Magnesium (mg L^{-1})	27.25	12.79
Copper (mg L^{-1})	0.030	0.023
Manganese (mg L^{-1})	0.033	<0.011
Zinc (mg L^{-1})	0.215	0.107
Chloride (mg L^{-1})	39.88	23.89
Sodium (mg L^{-1})	44.80	22.16
pH	7.78	6.52
Electrolyte Conductivity (mS cm^{-1})	5.11	3.62
Alkalinity (mg L^{-1})	213.91	127.83

was covered with a cone-shaped structure made from a plastic bottle to prevent drift or contamination of the control plants. Foliar application followed the recommendation for the vegetable, according to AgraForum (Supplier), with CibuSil applied at an 8 µL L^{-1} solution and AgraAlgen at a 5 µL L^{-1} solution.

Data collection: Data collection took place four weeks post-emergence as a once-off assessment. In this study, germination was assessed based on seedling emergence. The following seedling parameters were measured: seedling germination percentage (%), seedling vigour index (%), seedling height (cm), number of branches, leaf length (cm), leaf area (cm^2), stem diameter (mm), foliar fresh mass (FFM [g]), root length (cm), and fresh root mass (FRM [g]).

Seedling height, FFM, stem diameter, leaf area and FRM: Seedling height was measured from the top of the soil surface to the apical meristem using a measuring tape (Stanley, RSA) (Pérez-Harguindeguy *et al.* 2016). After cutting the seedling stems at the rooting medium surface, the following destructive measurements were taken: (a) the FFM mass (g), measured with a Denver Instrument scale (UK); (b) stem diameter, measured with a vernier caliper from 1 cm above the cotyledons from all the seedlings per treatment unit; and (c) leaf area, measured from all seedling leaves per treatment combinations using a leaf area meter (Li-Cor LI-3100C), as described by Pan *et al.* (2020).

Leaf length and number of branches per plant: Leaf length was measured along the leaf axis of the longest leaf from all the plants per treatment combinations, as described by Lee *et al.* (2022). The number of branches was determined by counting the final developed branches from all plants per treatment unit (Peixoto *et al.* 2017).

Root length and fresh root mass: The seedlings were removed manually from the seedling trays, and the shoot and root lengths of the seedlings were measured with a precision steel ruler (Vivek and Duraisamy 2017; Zhang *et al.* 2018).

Seedling germination percentage and vigour index: The total germination percentage was determined following Scott *et al.* (1984):

Table 3. Photomorphogenesis and biostimulant treatments (6 x 3 factorial design) for tomato seedlings

Photomorphogenesis combined with biostimulant treatments	Irrigation interval
S0-CS-I1	Once
S0-CS-I2	Twice
S0-CS-I3	Thrice
S0-AA-I1	Once
S0-AA-I2	Twice
S0-AA-I3	Thrice
S2-CS-I1	Once
S2-CS-I2	Twice
S2-CS-I3	Thrice
S2-AA-I1	Once
S2-AA-I2	Twice
S2-AA-I3	Thrice
S4-CS-I1	Once
S4-CS-I2	Twice
S4-CS-I3	Thrice
S4-AA-I1	Once
S4-AA-I2	Twice
S4-AA-I3	Thrice

S0 = Normal/0-day post-emergence darkness duration exposure, S2 = 2 days post-emergence darkness duration exposure, S4 = 4 days post-emergence darkness duration exposure, CS = CibuSil, AA = AgraAlgen, I1 = Irrigation once, I2 = Irrigation twice, I3 = Irrigation thrice

$Gt\ (\%) = (n / N \times 100)$

Where *Gt* is the total germination; *n* is the number of germinated seeds at the end of the experiment, and *N* is the total number of seeds.

The seedling vigour index was determined based on Rahnama-Ghahfarokhi and Tavakkol-Afshari (2007) and Steiner (1990):

$VI = Ls \times Gt/100$

Where *VI* is the vigour index, *Ls* is the mean of seedling length, and *Gt* is the germination percentage.

Statistical analysis: The experimental data from this trial were analyzed using the General Linear Model (GLM) in SAS statistical software version 9.2 (2008) to perform an analysis of variance (ANOVA). The ANOVA was calculated and averaged per multipot, containing six cells with independent seedlings, and was replicated three times. Duncan Multiple Range Test (DMRT) was applied to assess significant differences between variants. Statistical significance between treatment means was considered at the 0.05 probability level ($P<0.05$). DMRT was preferred over Tukey HSD due to its higher statistical power and stepwise comparison procedure, which enhances the detection of significant differences among treatments and improves grouping and ranking of treatment means under irrigation-frequency and post-emergence darkness stress conditions.

Results and discussion

The study indicated notable differences in the effects of the main factors (post-emergence darkness duration combined with biostimulants and irrigation intervals) and their interactions on germination and growth parameters measured in the tomato seedlings at harvest (Table 4). Fig. 1 illustrates the tomato seedlings at the end of the experiment, ready for transplant (Table 4). Parameters such as seedling germination, seedling vigour index, leaf area, and root length were significantly affected by the combinations of post-emergence darkness duration with biostimulants; while significant interactions were observed between post-emergence darkness duration for seedlings and the irrigation intervals on seedling height, leaf length, number of branches, stem diameter, FFM and FRM. No significant interaction was recorded between post-emergence darkness duration and irrigation interval on seedling germination and vigour index.

Specifically, total germination was significantly higher at 0 days of post-emergence darkness duration combined with CibuSil, and at 0, 2, and 4 days of post-emergence darkness duration combined with AgraAlgen, followed by 2 days of post-emergence darkness duration combined with CibuSil. Germination was significantly lower when post-emergence darkness duration was prolonged to four days in combination with CibuSil, compared to other treatments (Table 4). Overall, total germination decreased with increasing duration of post-emergence darkness duration under Cibusil treatment.

The results showed that seedling vigour index (SVI) significantly improved in tomato seedlings exposed to 0 days of post-emergence darkness duration with CibuSil treatment and 4 post-emergence darkness duration days with AgraAlgen treatment compared to CibuSil-treated seedlings exposed to 2 and 4 days of post-emergence darkness duration (Table 4). Seed germination and the vigour of young seedlings are highly responsive to the environmental cues that kickstart metabolism during the earliest stages of growth. Conditions such as soil moisture, temperature, oxygen availability, light exposure, and the chemical composition of the germination medium strongly influence whether seeds germinate (Ors *et al.* 2021; Zahra *et al.* 2023). In this study, the differences we observed in germination rates and vigour index across treatments likely reflect how these external factors interact with the physiological processes that control seed metabolism and embryo development.

When seeds germinate, sufficient water and oxygen enable enzymes to mobilize stored reserves, supporting radicle emergence and the establishment of the seedling. If conditions are poor or seeds remain in incubation for too long, however, metabolic stress and depletion of internal reserves can reduce viability. This helps explain why some seeds became nonviable under extended incubation, a result that aligns with Poštić *et al.* (2020), who reported dead tomato seeds after extending incubation to 14 days.

Generally, biostimulants, particularly seaweed extracts, may improve germination by activating hormonal pathways involved in early growth. Seaweed products contain bioactive molecules such as cytokinins, auxins and betaines that can promote cell division and metabolic activity during the seedling's first stages. Reis *et al.* (2020) similarly found that brown seaweed extract improved germination of aged seeds and enhanced seedling vigour. Thus, the gains in vigour seen here can plausibly be attributed to these compounds stimulating early metabolic processes and supporting stronger seedling establishment.

Tomato seedling height, stem diameter, and branching were closely associated and reflected contrasting growth responses to post-emergence darkness. Prolonged darkness, particularly when combined with CibuSil or AgraAlgen and frequent irrigation, promoted shoot elongation, resulting in taller seedlings. However, increased height was accompanied by reduced stem diameter and branching, indicating a shift in growth towards stem elongation rather than structural development. Thinner stems suggest reduced mechanical strength and lower capacity to support subsequent vegetative growth, while fewer branches reflect suppressed lateral bud outgrowth and reduced canopy development. In contrast,



Fig. 1. Photographic representation of tomato seedlings (Central University of Technology, Free State).

seedlings exposed to little or no post-emergence darkness developed thicker stems and more branches, particularly when treated with CibuSil, indicating greater biomass allocation to structural tissues and lateral growth. Collectively, these traits demonstrate that increased seedling height alone is not indicative of superior growth; rather, a combination of moderate height, greater stem diameter, and enhanced branching represents a more balanced morphological phenotype associated with improved seedling vigour, structural integrity, and transplant quality. The three parameters are discussed below.

At the highest irrigation interval (3 times daily), both CibuSil and AgraAlgen treatments increased the height of tomato seedlings exposed to 4 days of post-emergence darkness, outperforming all other treatment combinations; however, this may result in etiolation, subsequently affecting seedling quality. However, in this study, as illustrated in Fig. 1, seedings were ultimately similar to those exposed to prolonged post-emergence darkness duration, indicating a marginal quality. Additionally, CibuSil-treated seedling height was higher than AgraAlgen-treated seedling height at 2 days of post-emergence darkness duration. A significant interaction among irrigation interval, post-emergence darkness duration, and biostimulant type was observed for seedling height (P=0.0001). Seedling height in our trial seems to reflect the physiological shifts that occur when plants experience extended darkness after emergence. In prolonged dark conditions, seedlings typically show etiolation: stems elongate rapidly, leaves remain small, and the plant grows upward in search of light. Hormones such as auxins and gibberellins drive this response by promoting cell elongation under lowlight conditions. The taller growth recorded in seedlings kept in the dark likely represents an adaptive

attempt to reach light, a pattern also reported for tomatoes by Kalaitzoglou *et al.* (2019). In nursery practice, these elongated stems often need support or corrective handling once light is restored; mechanical support or trellising can help straighten curved stems (Ramanjineyulu *et al.* 2024). Combining CibuSil with appropriate irrigation may further aid recovery by helping seedlings maintain turgor and strengthen cell walls, supporting more robust recovery when normal light returns.

For stem diameter, both CibuSil and AgraAlgen treatments in the absence of post-emergence darkness duration (at 0 days), along with AgraAlgen treatment at 2 days of post-emergence darkness

Table 4. The effects of seaweed-based *(*AgraAlgen) and CibuSil biostimulants on germination and growth parameters of tomato seedlings subjected to different post-emergence darkness duration and irrigation intervals

Treatment parameters and output labels	Gt (%)	VI	SeedlingH (cm)	StemDia (mm)	NumBr	LeafL (cm)	LeafA (cm^2)	FFM (g)	RootL (cm)	FRM (g)
Post-emergence darkness duration combined with biostimulant										
Normal (0) post-emergence darkness duration with CibuSil	93.75^{a}	7.66^{a}	8.17^{bc}	8.22^{a}	5.17^{a}	6.31^{a}	39.25^{a}	3.66^{a}	11.71^{a}	0.71^{a}
Normal (0) post-emergence darkness duration with AgraAlgen	94.44^{a}	7.26^{ab}	7.69^{c}	8.22^{a}	4.43^{b}	6.05^{a}	35.33^{ab}	3.07^{b}	10.76^{ab}	0.71^{b}
Two post-emergence darkness durations with CibuSil	75.79^{b}	6.48^{b}	8.55^{b}	7.44^{ab}	4.48^{b}	5.34^{b}	31.78^{bc}	2.22^{c}	10.18^{abc}	0.64^{bc}
Two post-emergence darkness durations with AgraAlgen	92.56^{a}	7.16^{ab}	7.74^{c}	8.11^{a}	4.46^{b}	5.36^{b}	35.33^{ab}	2.88^{b}	10.91^{a}	1.17^{a}
Four post-emergence darkness duration with CibuSil	61.00^{c}	6.13^{bc}	10.05^{a}	7.11^{ab}	4.30^{b}	4.71^{c}	31.44^{bc}	1.34^{d}	9.17^{bc}	0.33^{d}
Four post-emergence darkness duration with AgraAlgen	85.11^{ab}	8.64^{a}	10.15^{a}	6.67^{b}	4.39^{b}	4.37^{c}	27.78^{c}	1.78^{cd}	8.68^{c}	0.38^{cd}
F-value	7.47	8.58	42.97	2.84	12.08	29.18	3.28	21.32	4.08	8.85
P-value	0.01	0.02	0.0001	0.03	0.0001	0.0001	0.02	0.01	0.01	0.01
LSD	14.07	0.91	0.50	1.12	2.03	0.39	6.24	0.54	1.61	0.29
Irrigation Interval										
Once a day (1)	83.29^{a}	6.88^{a}	8.26^{b}	7.67^{a}	4.64^{a}	5.12^{b}	30.17^{b}	1.94^{b}	10.47^{a}	0.38^{c}
Twice a day (2)	86.94^{a}	7.47^{a}	8.59^{b}	7.67^{a}	4.49^{a}	5.16^{b}	33.71^{ab}	2.77^{a}	9.62^{a}	0.99^{a}
Thrice a day (3)	80.50^{a}	7.53^{a}	9.36^{a}	7.56^{a}	4.48^{a}	5.73^{a}	36.28^{a}	2.67^{a}	10.60^{a}	0.60^{b}
F-value	0.89	0.93	23.70	0.05	1.99	11.92	4.14	9.41	1.77	19.04
P-value	0.42	0.39	0.0001	0.95	0.15	0.01	0.02	0.01	0.19	0.01
LSD	9.94	0.82	0.34	0.79	0.19	0.28	4.41	0.38	1.14	0.21
Post-emergence darkness duration x Irrigation Interval										
Norm. day x AgraAlgen-1	100.00^{a}	6.33^{a}	7.17^{g}	9.00^{ab}	4.44^{bc}	5.89^{abcd}	29.67^{a}	2.46^{cdef}	10.56^{a}	0.43^{bcde}
Norm. day x AgraAlgen-2	89.00^{a}	6.29^{a}	8.25^{defg}	8.33^{abc}	4.31^{bc}	5.75^{abcde}	35.00^{a}	2.97^{bcde}	9.56^{a}	0.82^{bc}
Norm. day x AgraAlgen-3	94.33^{a}	5.38^{a}	7.65^{fg}	7.33^{abcde}	4.53^{b}	6.50^{ab}	41.33^{a}	3.78^{ab}	12.17^{a}	0.89^{b}
Norm. day x CibuSil-1	77.67^{a}	6.21^{a}	7.61^{fg}	8.33^{abc}	5.17^{a}	6.50^{ab}	39.67^{a}	2.38^{defg}	11.95^{a}	0.27^{cde}
Norm. day x CibuSil-2	89.00^{a}	7.87^{a}	7.60^{fg}	8.67^{ab}	5.28^{a}	6.08^{abc}	37.67^{a}	3.39^{abcd}	11.42^{a}	0.89^{b}
Norm. day x CibuSil-3	94.33^{a}	6.65^{a}	8.50^{defg}	7.67^{abcde}	5.08^{a}	6.67^{a}	45.33^{a}	4.27^{a}	11.76^{a}	0.97^{b}
Two days x AgraAlgen-1	88.67^{a}	6.84^{a}	7.28^{g}	8.00^{abcd}	4.38^{bc}	5.03^{defg}	31.67^{a}	2.11^{defg}	11.41^{a}	0.52^{bcde}
Two days x AgraAlgen-2	100.00^{a}	7.37^{a}	7.67^{fg}	9.33^{a}	4.55^{b}	5.45^{cde}	40.00^{a}	3.68^{abc}	10.22^{a}	2.25^{a}
Two days x AgraAlgen_3	89.00^{a}	6.76^{a}	8.28^{defg}	7.00^{bcde}	4.44^{bc}	5.61^{bcde}	34.33^{a}	2.85^{bcde}	11.08^{a}	0.73^{bcd}
Two days x CibuSil-1	77.67^{a}	6.87^{a}	8.62^{def}	9.00^{ab}	5.29^{a}	5.39^{cdef}	30.00^{a}	2.26^{defg}	10.99^{a}	0.67^{bcde}
Two days x CibuSil-2	83.00^{a}	6.73^{a}	7.60^{fg}	6.00^{de}	3.93^{c}	5.00^{defg}	28.00^{a}	2.18^{defg}	8.47^{a}	0.76^{bcd}
Two days x CibuSil-3	66.67^{a}	7.47^{a}	9.42^{cd}	7.33^{abcde}	4.21^{bc}	5.64^{bcde}	37.33^{a}	2.22^{defg}	11.09^{a}	0.49^{bcde}
Four days x AgraAlgen-1	89.00^{a}	6.84^{a}	9.14^{de}	5.67^{e}	4.39^{bc}	3.81^{h}	25.00^{a}	1.44^{fgh}	8.64^{a}	0.24^{cde}
Four days x AgraAlgen-2	83.00^{a}	6.78^{a}	10.80^{ab}	6.33^{cde}	4.47^{bc}	4.47^{fgh}	28.67^{a}	2.12^{defg}	8.73^{a}	0.50^{bcde}
Four days x AgraAlgen-3	83.33^{a}	7.87^{a}	10.53^{bc}	8.00^{abcd}	4.31^{bc}	4.82^{efg}	29.67^{a}	1.77^{efgh}	8.66^{a}	0.38^{bcde}
Four days x CibuSil-1	50.00^{a}	6.84^{a}	8.81^{def}	6.00^{de}	4.19^{bc}	4.08^{gh}	25.00^{a}	0.60^{h}	9.31^{a}	0.15^{de}
Four days x CibuSil-2	77.67^{a}	6.76^{a}	9.57^{cd}	7.33^{abcde}	4.42^{bc}	4.90^{efg}	39.67^{a}	2.28^{defg}	9.37^{a}	0.74^{bcd}
Four days x CibuSil-3	55.33^{a}	6.89^{a}	11.78^{a}	8.00^{abcd}	4.30^{bc}	5.14^{cdef}	29.67^{a}	1.13^{hg}	8.84^{a}	0.11^{e}
F-value	0.97	0.34	7.05	3.08	4.09	2.15	1.92	2.38	0.59	4.45
P-value	0.48	0.69	0.0001	0.01	0.01	0.05	0.08	0.03	0.81	0.01
LSD	24.45	4.69	0.87	1.94	0.45	0.68	10.85	0.94	2.80	0.50

Means (are based on three replications; each replication consisted of one multipot containing six seedlings) followed by the same letter in the same column are statistically non-significant (Duncan's Multiple Range Test, $P<0.05$). Norm. day x AgraAlgen-1 = Normal (0) post-emergence darkness duration with AgraAlgen x Once a day; Norm. day x AgraAlgen-2 = Normal (0) post-emergence darkness duration with AgraAlgen x Twice a day; Norm. day x AgraAlgen-3 = Normal (0) post-emergence darkness duration with AgraAlgen x Thrice a day; Norm. day x CibuSil-1 = Normal (0) post-emergence darkness duration with CibuSil x Once a day; Norm. day x CibuSil-2 = Normal (0) post-emergence darkness duration with CibuSil x Twice a day; Norm. day x CibuSil-3 = Normal (0) post-emergence darkness duration with CibuSil x Thrice a day; 2 days x AgraAlgen-1 = 2 post-emergence darkness duration with AgraAlgen x Once a day; 2 days x AgraAlgen-2 = 2 post-emergence darkness duration with AgraAlgen x Twice a day; 2 days x AgraAlgen-3 = 2 post-emergence darkness duration with AgraAlgen x Thrice a day; 2 days x CibuSil-1 = 2 post-emergence darkness duration with CibuSil x Once a day; 2 days x CibuSil-2 = 2 post-emergence darkness duration with CibuSil x Twice a day; 2 days x CibuSil-3 = 2 post-emergence darkness duration with CibuSil x Thrice a day; 4 days x AgraAlgen-1 = 4 post-emergence darkness duration with AgraAlgen x Once a day; 4 days x AgraAlgen-2 = 4 post-emergence darkness duration with AgraAlgen x Twice a day; 4 days x AgraAlgen-3 = 4 post-emergence darkness duration with AgraAlgen x Thrice a day; 4 days x CibuSil-1 = 4 post-emergence darkness duration with CibuSil x Once a day; 4 days x CibuSil-2 = 4 post-emergence darkness duration with CibuSil x Twice a day; 4 days x CibuSil-3 = 4 post-emergence darkness duration with CibuSil x Thrice a day; Gt = Total Germination; VI = Seedling Vigour Index = SeedlingH = Seedling Height; StemDia = Stem Diameter; NumBr = Number of Branches; LeafL = Leaf Length; LeafA: Leaf Area; FFM = Foliar Fresh Mass; RootL = Root Length; FRM = Fresh Root Mass.

duration, produced thicker stems than AgraAlgen-treated seedlings at 4 days of post-emergence darkness duration (Table 4). While irrigation interval alone did not significantly affect stem diameter, a significant interaction between post-emergence darkness duration and irrigation interval was observed (P=0.01). CibuSil-treated seedlings exposed to 0 days of post-emergence darkness duration had the highest number of branches among all treatment combinations (Table 4). Although irrigation interval alone did not exert a significant effect on the number of branches, the interaction between post-emergence darkness duration and irrigation interval was significant (P=0.01). Stem thickness is a useful indicator of seedling sturdiness and future capacity to support vegetative growth. In our study, stem diameter varied with early environmental conditions, especially the length of the postemergence dark period. Extended darkness tends to favour vertical growth over radial thickening, producing thinner stems because resources are channelled into elongation rather than structural reinforcement. This trade-off has been observed in tomato seedlings (García-Caparrós *et al.* 2020) , where prolonged post-emergence darkness duration reduced stem girth. These results underscore the need to manage light exposure carefully in nurseries to promote balanced stem development.

Branching in tomato seedlings is shaped by environmental cues and the hormonal control of apical dominance. During extended dark periods, plants often prioritise upward growth and suppress lateral bud activation, thereby reducing branching. This shift is largely governed by the auxin-cytokinin balance, which regulates lateral bud outgrowth. Our data suggests that CibuSil may help sustain vigour and branching under these conditions, possibly by supporting nutrient uptake and stress resilience. Irrigation frequency also influenced morphology, likely through effects on plant water status and nutrient mobility in the substrate. Similar links between watering regime and branching have been reported by Gu *et al.* (2024), who found that less frequent irrigation was associated with reduced lateral development.

Leaf length was significantly influenced by the highest irrigation interval (thrice daily) as well as the interaction between post-emergence darkness duration and irrigation interval (Table 4). CibuSil and AgraAlgen treatments with 0 days of post-emergence darkness duration produced the longest leaves, followed by CibuSil and AgraAlgen with 2 days of post-emergence darkness duration. The shortest leaf lengths were recorded in CibuSil and AgraAlgen treatments with 4 days of post-emergence darkness duration. Overall, a consistent downward trend in leaf lengths was observed with increasing duration of post-emergence darkness under both CibuSil and AgraAlgen treatments.

CibuSil- and AgraAlgen-treated seedlings with 0 days of post-emergence darkness duration, as well as AgraAlgen-treated seedlings with 2 days of post-emergence darkness duration, produced larger leaf areas compared to seedlings exposed to 4 days of post-emergence darkness duration and treated with AgraAlgen (Table 4). Additionally, CibuSil-treated seedlings with 0 days of post-emergence darkness duration had higher leaf areas than CibuSil-treated seedlings with 2 days of post-emergence darkness duration. The leaf area was significantly better where the seedlings received more than two irrigations interval (Table 4). However, irrigation interval with post-emergence darkness duration and biostimulant treatments were not significant.

The findings of this study showed that fresh foliar mass (FFM) was significantly influenced by frequent irrigation (twice and thrice daily) and by the interaction between post-emergence darkness duration and irrigation interval (Table 4). Fresh foliar mass was greatest in CibuSil-treated seedlings exposed to 0 days of post-emergence darkness duration, followed by AgraAlgen-treated seedlings under both 0 and 2 days of post-emergence darkness duration. CibuSil-treated seedlings FFM at 2 days of post-emergence darkness duration outperformed CibuSil-treated seedlings FFM at 4 days of post-emergence darkness duration. Overall, FFM decreased with increasing duration of post-emergence darkness duration, particularly under CibuSil treatment.

Interestingly, root length was generally higher under shorter post-emergence darkness duration exposure. Specifically, longer roots were observed in seedlings treated with CibuSil at 0 days of post-emergence darkness duration and AgraAlgen at 2 post-emergence darkness duration-days (Table 4). In contrast, both treatments resulted in shorter roots when applied after 4 days of post-emergence darkness duration. Even AgraAlgen at 0 post-emergence darkness duration days produced longer roots than AgraAlgen at 4 days.

Fresh root mass was significantly influenced by irrigation (twice daily) and by the interaction between post-emergence darkness duration and irrigation interval (Table 4). Seedling root development was strongly influenced by both biostimulant treatment and the duration of post-emergence darkness. CibuSil-treated seedlings with 0 days of post-emergence darkness duration exhibited the highest fresh root mass overall, while AgraAlgen-treated seedlings at 2 days maintained relatively high root mass under moderate dark stress. In contrast, seedlings subjected to 4 days of post-emergence darkness duration consistently showed reduced root mass, with the decline being more pronounced in CibuSil treatments. Notably, AgraAlgen-treated seedlings at 0 days produced greater root mass than both CibuSil- and AgraAlgen-treated seedlings at 4 days, and within CibuSil treatments, seedlings exposed to 2 days of post-emergence darkness duration outperformed those at 4 days. These findings indicate that post-emergence darkness duration negatively affects root development, and the magnitude of decline depends on the biostimulant applied. The interaction between treatment type and timing is therefore critical: AgraAlgen appears to support root growth more effectively under moderate dark stress, whereas CibuSil is most effective under no dark stress. This highlights the importance of optimizing both biostimulant choice and post-emergence darkness duration to maximize seedling performance.

Fig. 1 shows the differences in seedling height between the treatments. The tallest seedlings were recorded, with seedlings treated with CibuSil and irrigated three times a day; this occurred in plants kept four days longer after the normal (0) germination period, as illustrated in Table 4. Interestingly, irrigation frequency was found to be the factor affecting seedling height, especially when CibuSil was used, with plants remaining in post-emergence darkness duration for 4 days longer. When similar treated plants were compared based on the biostimulant combination, CibuSil performed better compared to AgraAlgen. Moreover, as shown in Fig. 1, other factors that could have contributed to taller seedlings, besides irrigation frequency, are phototropism and etiolation. Seedlings were kept in a dark room to facilitate emergence; however, due to prolonged post-emergence darkness duration over 4 days, they were etiolated, resulting in elongated, curved stems and affecting seedling quality. Nonetheless, this

may result in etiolation, which could subsequently affect seedling quality. As illustrated in Fig. 1, seedlings were ultimately similar to those exposed to prolonged post-emergence darkness duration, indicating a marginal quality. The visual assessment of seedlings (Fig. 1) corroborates the conclusion that prolonged post-emergence darkness reduced seedling quality rather than promoting desirable growth. Although seedlings exposed to extended darkness exhibited greater shoot elongation, this response was characteristic of etiolation and was associated with reduced morphological quality, including thinner stems and diminished branching. Therefore, the observed increase in seedling height should be interpreted as a stress-induced adaptive response to light deprivation rather than an indicator of improved seedling vigour or transplant quality.

Increasing the irrigation frequency to three times a day on the seedlings, for which post-emergence darkness duration was prolonged for two days, significantly increased the stem diameter of tomato seedlings (Table 4). This occurred when AgraAlgen, two days of prolonged post-emergence darkness duration, and a combination were administered to plants; however, this was not significantly different from CibuSil administration with an at least once-a-day irrigation frequency. Therefore, this suggests that it is beneficial to administer the CibuSil combination to seedlings that have been prolonged for 2 days in the post-emergence darkness duration state. Interestingly, it could be observed that increasing the number of days post-emergence from two to four days resulted in thinner and curvy stems, irrespective of the biostimulant application (Fig. 1), this could be associated with the results in Table 4. However, this study recommends that both biostimulants be administered in combination with prolonged post-emergence irrigation for seedlings irrigated at least twice a day. Table 4 shows that further increasing the irrigation frequency from twice to three times a day results in a thinner stem diameter of seedlings.

The seedlings were grown for four weeks, a standard period followed by the tomato nurseries in South Africa (Ngcobo *et al.* 2024). At four weeks, a difference between treatments in the number of branches was observed as seedlings developed (Fig. 1). As illustrated in Table 4, applying CibuSil-two days post seedling emergence combination on seedlings irrigated once showed to increase in the number of branches compared to AgraAlgen-two days prolonged post-emergence darkness duration combination. It was interesting to note a gradual decrease in the number of branches as irrigation frequency increased from twice a day to three times.

The leaf length varied across the treatments, and this can be observed in Fig. 1 depiction. Table 4 further shows a significant (P<0.04) interaction between the main factors, the biostimulant-days after seedling emergence combination and the irrigation interval. As illustrated in Table 4, longer leaves were observed in plants treated with CibuSil and irrigated twice a day. However, this was not significantly different from seedlings treated with AgraAlgen and irrigated twice daily. All these occurred during a normal post-emergence darkness duration germination period, in combination with the application of biostimulants. The seedling leaf area was significantly affected by the main factors, days post-emergence (P<0.02) and irrigation frequency (P<0.02). Extending the days after emergence as a form of stress on tomato seedlings in the state of post-emergence darkness duration did not affect leaf area, irrespective of the combination of biostimulant type and irrigation interval (Table 4). On the other hand, broader leaves were recorded when the irrigation interval was significantly increased from once a day to three times a day. As illustrated in Table 4, seedlings irrigated three times had broader leaves than those irrigated once a day, irrespective of the irrigation interval. Early leaf expansion depends strongly on light, water and nutrient availability. When seedlings remain in darkness, energy is diverted to stem elongation and leaf expansion is reduced, explaining the shorter leaves observed under extended dark treatments. This allocation trade-off can lower photosynthetic potential if light exposure is not restored in time. Beyond light, irrigation and biostimulant treatments can influence leaf growth by improving water relations and nutrient availability; for example, foliar silicon has been shown to increase leaf length and area under varying irrigation in squash (Salim *et al.* 2021), likely by strengthening cell walls and improving tolerance to physiological stress.

Leaf expansion relies on cell division and enlargement, processes that are constrained when metabolic resources are redirected toward stem elongation during post-emergence darkness duration. Yuan *et al.* (2025) reported , with a linear decrease in leaf area in young tomato plants resulting from increasing the number of light-limitation cycles. Water availability is also crucial: adequate irrigation maintains cell turgor needed for expansion. In this study, increasing irrigation from once to twice daily supported leaf expansion without further gains from more frequent watering, a pattern consistent with Puértolas and Dodd (2022) and Zhang *et al.* (2024), who found that moderate increases in irrigation frequency improved leaf area and gas exchange in greenhouse tomatoes.

As depicted in Table 4, the highest FFM (P<0.03) was recorded when seedlings germinated under normal conditions (four to six weeks; this study was terminated after four weeks) and were irrigated twice before transplant (Maboko and Du Plooy 2014). When seedlings were held in the post-emergence darkness duration, post-seedling-emergence combination for 2 days, a gradual increase in FFM was observed with increasing irrigation frequency, but only when AgraAlgen was used. Nonetheless, it is recommended that the seedlings be prolonged for two days of post-emergence darkness duration in combination with AgraAlgen administration, and the irrigation be kept to twice a day until transplant. Fresh foliar mass reflects the living leaf biomass and is a useful indicator of vegetative growth. Biomass accumulation depends on water supply, nutrient uptake and hormonal regulation. Seaweedderived biostimulants can boost biomass by improving stress tolerance and stimulating metabolic activity; Al-Bayati *et al.* (2020) found that seaweed extract combined with higher irrigation increased fresh leaf mass in eggplant, a same family as tomatoes, and Sohrabi *et al.* (2024) reported similar gains in foliar and stem biomass with plant extracts under frequent irrigation. These findings suggest biostimulants can enhance water and nutrient use efficiency, supporting steadier vegetative growth when irrigation is adequate.

Fig. 1 depicts a qualitative photographic representation of root length, and a significant (P<0.01) difference is evident across treatments. No significant differences could be recorded on seedlings sown under the normal state of post-emergence darkness duration and those with at least two more days of prolonged post-emergence under the state of post-emergence darkness duration, irrespective of the combination with the

biostimulant administration. However, when the days were extended beyond 2 during post-emergence darkness duration, root length was significantly shorter; this was true regardless of the combination with biostimulant administration.

A significant interaction was observed on the FRM, attributed to increased irrigation frequency (Table 4). Compared with once- and twice-a-day irrigation frequencies, three-times-a-day irrigation significantly increased FRM in seedlings treated with AgraAlgen. This occurred in seedlings that emerged and persisted for 2 additional days during post-emergence darkness duration, in combination with the administered AgraAlgen. Interestingly, prolonging seedling exposure to post-emergence darkness duration conditions from 2 to 4 days, regardless of biostimulant application, resulted in significantly lower root mass.

Root development is essential for water and nutrient uptake, but early environmental stresses can shift resource allocation toward shoots at the expense of roots. Prolonged post-emergence darkness duration often leads to shorter roots because the plant prioritises shoot elongation to reach light; Yuan *et al.* (2025) observed this in tomato seedlings exposed to extended darkness cycles. Nevertheless, careful irrigation management and the use of biostimulants can mitigate these effects by improving nutrient mobility and water availability in the root zone. For example, Shang *et al.* (2023) showed that combining increased irrigation with seaweed liquid fertilizer enhanced both root and foliar mass in tomato seedlings grown in acidaffected soils, likely by improving nutrient uptake and water retention and thereby supporting greater fresh biomass accumulation.

Between the two tested products, CibuSil and AgraAlgen showed trait-specific responses: effective reductions in stress in tomato seedlings, increasing seedling vigour, leaf area, and root and shoot biomass. However, this study lacked a control for the biostimulants. While CibuSil promotes stem growth and branching, AgraAlgen is more effective at increasing root and foliar mass under the combined stress of prolonged post-emergence darkness duration exposure and varying irrigation intervals. This biostimulant comparative study concluded that the CibuSil application was the most effective for overall morphological improvements (height, branching, leaf area) under moderate irrigation intervals (one or two times a day), whereas AgraAlgen improved biomass and root health, particularly under extended post-emergence darkness duration conditions. Therefore, under the conditions of this study, CibuSil and AgraAlgen showed trait-specific effects on tomato seedling performance under variable irrigation frequency and post-emergence darkness duration. CibuSil was generally associated with greater seedling height, branching and leaf area under non- or moderately stressed conditions, whereas AgraAlgen better supported root development and fresh biomass under moderate post-emergence darkness stress. Prolonging post-emergence darkness to four days reduced seedling quality, particularly through etiolation and reduced root and foliar development. Because the study did not include an untreated control, the findings should be interpreted as a comparative response between the two biostimulants rather than definitive evidence of stress mitigation.. Nonetheless, this study suggests that combining these biostimulants with moderate irrigation intervals can improve tomato seedling resilience to environmental stresses.

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