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Exogenous application of proline and glucose on saline-stressed cucumber seedlings

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Abstract
Salinity is one of the abiotic stresses that can limit cucumber seedling production. It has detrimental effects on the growth and development of the seedlings including reverse osmosis, stunted growth and wilting. Research on the effects of proline and glucose on salt stressed plants has been done independently. This research therefore focused on the investigation of the effects of combining proline and glucose to mitigate saline stress in the early growth and development of cucumber seedlings. It therefore analysed and reported on the effects of exogenous application of proline and glucose on saline stressed cucumber seedlings. The variety used was Pointsett from Avanos Seeds Company. The cucumber seedlings were subjected to salinity stress of 2.5dS/m. Treatments were replicated three (3) times in a Completely Randomized Design (CRD). The treatments were: 0mM+0mmol/l; 10mM+50mmol/l; 20mM+50mmol/l and 30mM+50mmol/l. Data collected was subjected to one-way analysis of variance (ANOVA) and significance between means were determined using the least significant difference (LSD) test at P = 0.05% level. Results indicated linear increase in shoot diameter, shoot length and root length, root to shoot ratio, fresh weight and dry weight of seedlings, leaf length and leaf width. The results were presented in the form of clustered column bars, line graphs with trend lines showing linear equations and R^2 values, as well as treatment means tables which were indicating LSD values for the observed parameters. The conclusion made was that as the levels of combined proline and glucose increase, even at the lowest level, so do the observed parameters, indicating the positive impact of treatment on the growth and development of saline stressed cucumber seedlings. However, as the treatment concentrations used in this study did not reach an optimum level of combined proline and glucose to use, further research is required to unravel the optimum treatment level which can be recommended to cucumber producers.

Keywords: Cucumbers, proline, glucose, salinity stress, significant

Introduction
Soil salinity is a major environmental concern that affects the production of salt sensitive crops like cucumbers by restricting their growth and development. Just like other vegetable species, cucumbers succumb to various abiotic stresses including saline stress. According to Marium *et al.* (2019), cucumber is very sensitive to salt stress. Excessive salt content in soils seriously affects normal growth and development, posing a grim threat to commercial cucumber production (Liu *et al.* (2021) [10].

The higher the salt in the soils or irrigation water, the more growth and development of cucumbers is restricted. Al-Momany & Abu-Romman (2023) [3] affirms that at an EC of about 2.5 dSm-1 cucumber yield diminished by 13%. To achieve high yield and good quality cucumbers it is extremely imperative therefore to ensure that cucumber production is done in non-saline conditions. Existing literature shows that research on the effects of proline and glucose in ameliorating salt stress in plants has been done independently. This research therefore focused on finding out the influence of combining proline and glucose to mitigate saline stress in the early growth and development of cucumber seedlings.

Materials and methods
Study Site & Research Design
The research was carried out at Africa University (18.8968° S, 32.6013° E). A Completely Random Design (CRD) with four (4) treatments replicated 3 times was used. The treatments were exogenous proline & glucose at the following concentrations: A [0 mM + 0 mmol/l]; B [10 mM + 50 mmol/l]; C [20 mM + 50 mmol/l] and D [30 mM + 50 mmol/l]. The treatments were randomized as indicated in Table 1 below below.

Table 1: Experiment layout

A 1	D 2	C 3	B 4
D 5	C 6	B 7	A 8
B 9	A 10	D 11	C 12

Sampling and data collection
Systematic Sampling Method was used. Sample plants were picked out after every 3 plants in each plot. Data was collected on days 14, 21 and 28 of the experiment as follows.

Average Shoot length
Obtained by measuring the shoot length of each of the samples from the same treatment, using a 30cm ruler and calculating the mean

Average Root length
Obtained by measuring the root length of each of the samples of the same treatment, using a 30cm ruler and calculating the mean

Average Shoot diameter
Obtained by measuring the shoot diameter of each of the samples of the same treatment, using a Vernier calliper and calculating the mean

Average Fresh weight of Seedlings
Obtained by weighing each of the samples on a digital precision scale immediately after collecting them from the shade house, and calculating the mean

Average Dry weight of Seedlings
Obtained by weighing each of the samples on a digital precision scale after drying them up in an oven at 105 °C for 24 hours

Average Dry weight of Roots
Obtained by weighing the roots of the samples separated from the shoots, on a digital precision scale after drying them up in an oven at 105 °C for 24 hours.

Average Dry Weight of Shoots
Obtained by weighing the shoots of the samples separated from the roots, on a digital precision scale after drying them up in an oven at 105 °C for 24 hours.

Root/shoot ratio
Obtained by using the formula: dry weight of roots/dry weight shoot expressed as a decimal.

Average Leaf length
Obtained by measuring the leaf length of each of the samples using a 30cm ruler and calculating the mean.

Average Leaf width
Obtained by measuring the leaf width of each of the samples using a 30cm ruler and calculating the mean.

Data Analysis
Data collected was subjected to statistical analysis on GENSTAT (GEN532-2\BIN) using a one-way analysis of variance (ANOVA). Differences between means were determined using the least significant difference (LSD) test at P = 0.05 level.
The model used was.

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Where: Yij is the j^{th} observation of the ith treatment, μ is the population mean, τi is the treatment effect of the i^{th} treatment, and εij is the random error.

Results
Average Shoot Diameter
Figure 1 presents the effect of exogenous application of proline and glucose on the average shoot diameter of cucumber seedlings with varying concentrations of combined proline and glucose.

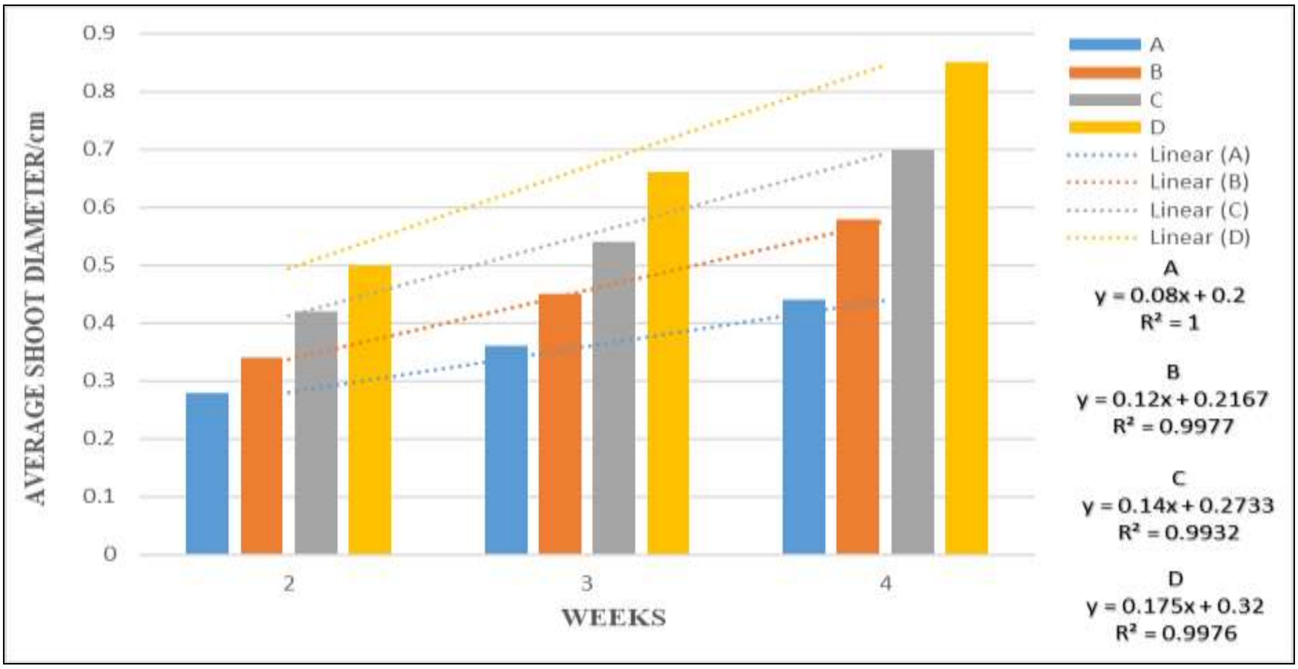

Fig 1: The Effect of Exogenous Application of Proline and Glucose on the Average Shoot Diameter of Cucumber Seedlings at week 2, 3 and 4

At week 2, treatment A seedlings had an average shoot diameter of 0.28 cm; followed by those from treatment B at 0.34 cm; treatment C at 0.42 cm and lastly those from treatment D at 0.52 cm.

At week 3, the seedlings from treatment A had the lowest average shoot diameter of 0.36 cm, followed by those from treatment B at 0.45 cm, treatment C at 0.54 cm and lastly those from treatment D at 0.66 cm.

At week 4, as shown in Figure 1, the seedlings from treatment A had an average shoot diameter of 0.44 cm, followed by those from treatment B at 0.58 cm; followed by those from treatment C at 0.70 cm and lastly those from treatment D at 0.85 cm.

Across week 2, 3 and 4, the average shoot diameter showed linear growth for each of the treatments as indicated by the four (4) trend lines for each of the treatments over time (Fig. 1). The linear equations and R^2 values for the trend lines are as indicated in Fig. 1.

Average Shoot Length

Figure 2 presents the effect of exogenous application of proline and glucose on the average shoot length of the cucumber seedlings with varying concentrations of combined proline and glucose

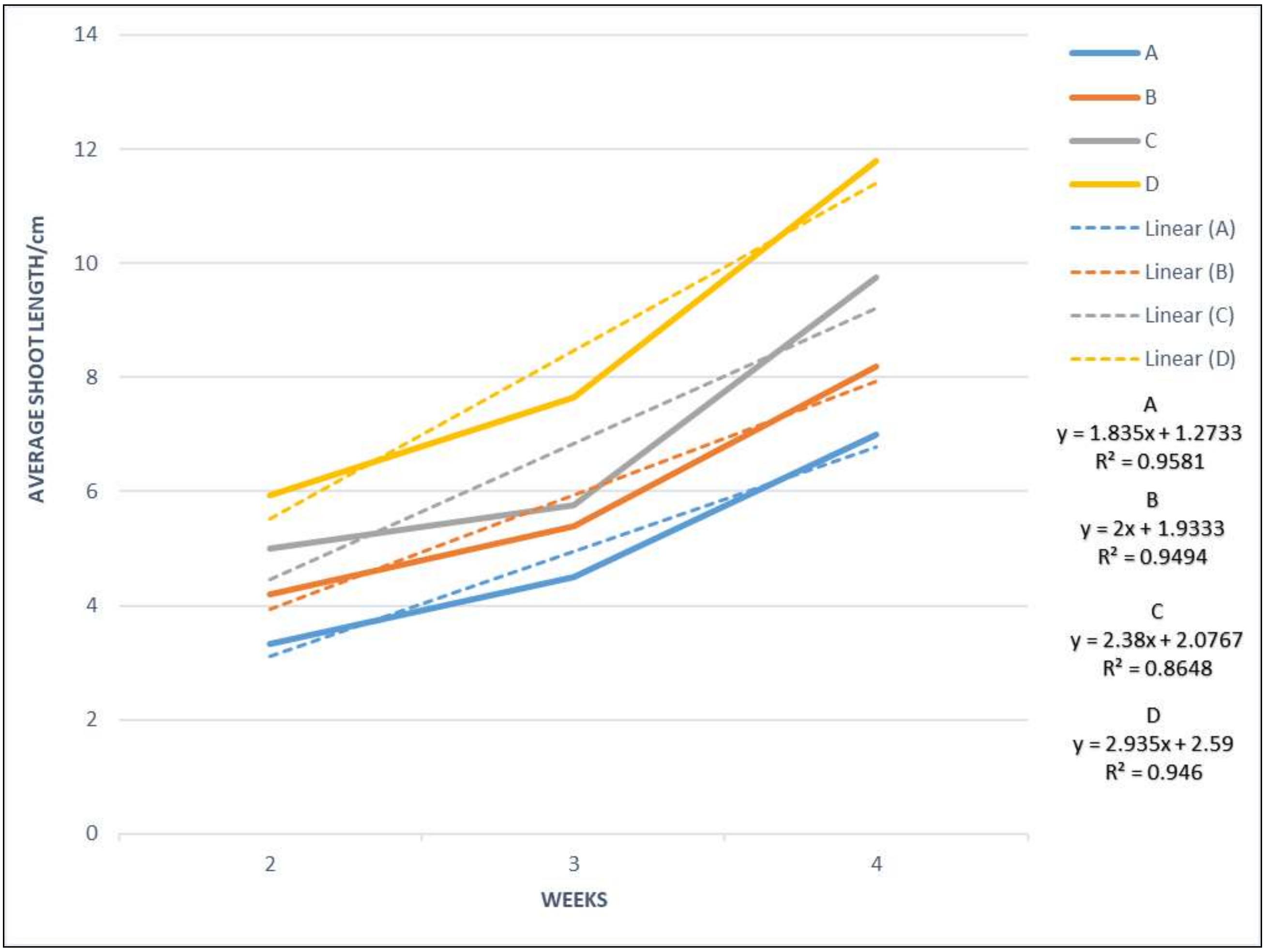

Fig 2: The Effect of Exogenous Application of Proline and Glucose on the Average Shoot Length of Cucumber Seedlings at week 2, 3 and 4

At week 2, seedlings from treatment A had an average shoot length of 3.50 cm; followed by those from treatment B at 4.20 cm; treatment C at 5.00 cm and lastly those from treatment D at 5.93 cm.

At week 3, the seedlings from treatment A had the lowest average shoot length of 4.50 cm, followed by those from treatment B at 5.40 cm, treatment C at 5.75 cm and lastly those from treatment D at 7.65 cm.

At week 4, the seedlings from treatment A had an average shoot length of 7 cm, followed by those from treatment B at 8.20 cm; treatment C at 9.76 cm and lastly those from treatment D at 11.80 cm (Fig 2).

Across week 2, 3 and 4, the average shoot length also showed straight line growth for each of the treatments as indicated by the four (4) trend lines for each of the treatments over time. The linear equations and R^2 values for the trend lines as indicated in Fig. 2.

Effect of Exogenous Application of Proline and Glucose on the Average Root Length

Figure 3 presents the effect of exogenous application of proline and glucose on the average root length of the cucumber seedlings with varying concentrations of combined proline and glucose

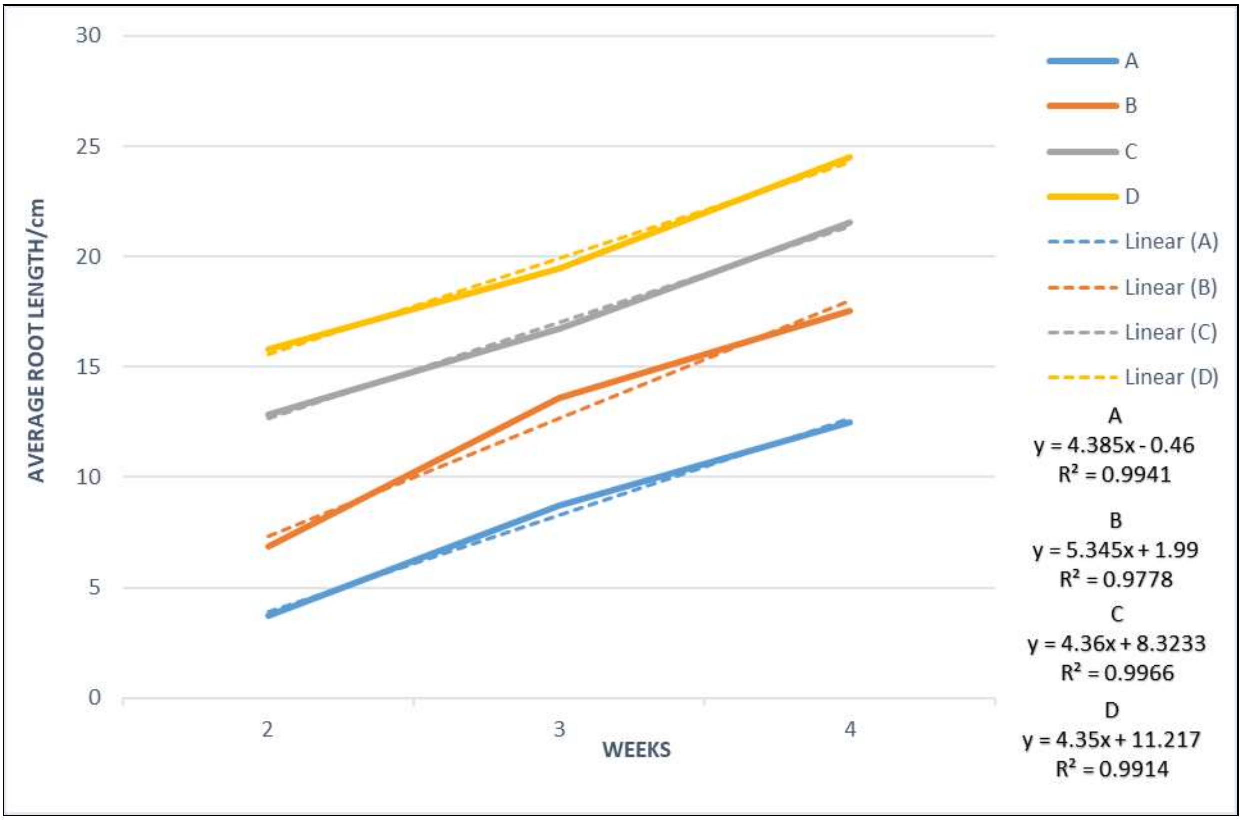

Fig 3: The Effect of Exogenous Application of Proline and Glucose on the Average Root Length of Cucumber Seedlings

At week 2, the seedlings from treatment A had an average root length at 3.73 cm; followed by those from treatment B at 6.87 cm; treatment C at 12.83 cm and lastly those from treatment D at 15.80 cm.

At week 3, the seedlings from treatment A had the lowest average root length of 8.70 cm, followed by those from treatment B at 13.61 cm, treatment C at 16.75 cm and lastly those from treatment D at 19.45 cm.

At week 4, the seedlings from treatment A had an average root length of 12.50 cm, followed by those from treatment B with 17.56 cm; treatment C with 20.25 cm and lastly those from treatment D with 24.50 cm.

Across week 2, 3 and 4, the average root length also showed incremental growth in a linear manner for each of the treatments. This can be seen on the graph as there are 4 trend lines for each of the treatments over time. The linear equation and R^2values for the trend lines are also indicated in Fig 3.

However, the optimum level of inclusion of proline and glucose for the above mentioned parameters was not reached in this study according to Fig 1, 2 and 3.

Treatment means and LSD values in week 2

Table 2 shows the results obtained at week 2 in the form of treatment means, LSD values and the significant differences between the treatments for root to shoot ratio; fresh-weight of seedlings; dry weight of seedlings; leaf length and leaf width.

Table 2: Treatment means and LSD'S IN WEEK TWO (2)

Growth parameter	A [0mM+0mmol/l]	B [10mM+50mmol/l]	C [20mM+50mmol/l]	D [30mM+50mmol/l]	LSD Value
Average Root to shoot ratio	0.13^{a}	0.27^{a}	0.42^{b}	0.56^{b}	0.1468
Average Fresh Weight of Seedlings	0.93^{a}	1.34^{b}	1.653^{c}	2.78^{d}	0.2766
Average Dry weight of seedlings	0.09^{a}	0.13^{a}	0.16^{a}	0.25^{ab}	0.1282
Average Leaf Length	3.07^{a}	4.13^{b}	5.61^{c}	6.73^{d}	0.4013
Average Leaf Width	2.93^{a}	3.97^{b}	5.33^{c}	6.80^{d}	0.3994

NB: Treatment means in the same row with different superscripts indicate significant differences between the treatments at $p<0.05$
Treatment means in the same row with at least a common superscript indicate insignificant differences between the treatments at $p>0.05$

As shown in Table 2 above, the treatment means for all the parameters were increasing in sequential progression with each treatment such that the lowest means for all parameters were recorded from cucumber seedlings from treatment A and the highest means recorded from the cucumber seedlings receiving treatment D.

Treatment means and LSD values in week 3

As indicated in Table 3, at week 3 there were significant differences between the root to shoot ratio; fresh-weight of seedlings; dry weight of seedlings; leaf length and leaf width.

Table 3: Treatment means and LSD Values

Growth parameter	A [0mM+0mmol/l]	B [10mM+50mmol/l]	C [20mM+50mmol/l]	D [30mM+50mmol/l]	LSD Value
Average Root to shoot ratio	0.14^{a}	0.32^{a}	0.53^{bc}	0.64^{bc}	0.2244
Average Fresh Weight of Seedlings	3.22^{a}	4.07^{ab}	5.48^{b}	7.73^{c}	0.900
Average Dry weight of seedlings	0.33^{a}	0.41^{b}	0.52^{c}	0.64^{d}	0.0893
Average Leaf Length	4.47^{a}	5.83^{b}	7.07^{c}	8.53^{d}	0.3438
Average Leaf Width	4.6^{a}	5.3^{b}	6.87^{c}	8.93^{d}	0.4580

NB: Treatment means in the same row with different superscripts indicate significant differences between the treatments at $p<0.05$
Treatment means in the same row with at least a common superscript indicate insignificant differences between the treatments at $p>0.05$

Treatment means (Table 3) for all the parameters studied were increasing in sequential progression with each treatment such that the lowest means for all parameters were recorded from cucumber seedlings from treatment A and the highest means recorded from the cucumber seedlings receiving treatment D.

Treatment means and LSD values in week 4.
Table 4 shows the results obtained at week 4 in the form of treatment means, LSD values and the significant differences between the treatments for root to shoot ratio; fresh-weight of seedlings; dry weight of seedlings; leaf length and leaf width.

Table 4: Treatment means and LSD values at week 4.

Growth parameter	A [0mM+0mmol/l]	B [10mM+50mmol/l]	C [20mM+50mmol/l]	D [30mM+50mmol/l]	LSD Value
Average Root to shoot ratio	0.20^{a}	0.35^{b}	0.57^{c}	0.70^{d}	0.0799
Average Fresh Weight of Seedlings	5.46^{a}	7.82^{b}	9.74^{c}	13.20^{d}	1.293
Average Dry weight of seedlings	0.67^{a}	0.73^{a}	0.84^{ab}	1.14^{b}	0.3864
Average Leaf Length	7.63^{a}	8.53^{b}	10.57^{c}	12.47^{d}	0.3805
Average Leaf Width	7.57^{a}	8.17^{b}	10.3^{c}	12.10^{d}	0.5854

NB: Treatment means in the same row with different superscripts indicate significant differences between the treatments at $p<0.05$. Treatment means in the same row with at least a common superscript indicate insignificant differences between the treatments at $p>0.05$.

As indicated, the treatment means for all the parameters were increasing in sequential progression with each treatment such that the lowest means for all parameters were recorded from cucumber seedlings from treatment A, and the highest means recorded from the cucumber seedlings with treatment D.

Fig 4: Cucumber seedlings (Treatment A) and (Treatment D) from week 4

Discussion

Average Shoot Diameter & Shoot Length of Cucumber Seedlings: The cucumber seedlings receiving treatment A had the smallest shoot diameter & shoot length because treatment A did not contain any proline nor glucose and therefore the cucumber seedlings were affected by the salinity stress. The seedlings lost water via reverse osmosis, causing photosynthesis and translocation to slow down, resulting in less photo assimilates. Reduced dry matter accumulation and partitioning, led to stunted shoot growth. Jenkins (2022) [7] asserted that high osmotic pressure between the root and the soil causes a slow reaction due to accumulation of Na^{+} and Cl^{-} in the leaves of the plant leading to a reduction in shoot growth. High level of proline (30 mM) and glucose resulted in large shoot diameter & shoot length because of the effectiveness of the two in ameliorating the effects of salinity on the cucumber seedlings. Shoot growth was also promoted the most in these seedlings. Proline acted as an osmoprotectants in the saline stressed cucumbers and helped to maintain water retention of the plant cells, such that they remained turgid, ensuring that water was not lost from the seedlings. Huang *et al.* (2010) observed that, the exogenous application of proline significantly alleviates the growth inhibition of plants induced by NaCl, and was accompanied by higher leaf relative water content and POD activity, higher proline and Cl^{-} contents, and lower MDA content and SOD activity. Exogenous glucose alleviated effects of salinity by being available for important processes like respiration, which produced more water, carbon dioxide and ATP needed by the salt stressed cucumber seedlings for growth. As the concentration of combined proline and glucose increased, the average shoot diameter and shoot length of the cucumber seedlings also increased. Hosseinifard *et al.* (2022) [6] affirm that, application of exogenous proline to plants exposed to stress increases growth and other physiological characteristics. Increased shoot diameter promoted better mechanical strength and stability of the seedlings, translocation of more water and nutrients in the plant body, as well as better water storage in the plant despite growing under salinity stress. Increased shoot length promoted faster rates of photosynthesis due to having more leaves on the shoots, providing a larger surface area for interception of solar radiation, resulting in more growth due to increased dry matter accumulation despite growing under salinity stress.

Average Root Length of Cucumber Seedlings: The cucumber seedlings which did not receive proline and glucose had the shortest root length because of the salinity stress which caused stunted root growth. This could have led to cellular damage of the root cells due to ion toxicity and dehydration, leading to inhibition of growth and development of the root system thus causing stunted root growth. According to Abdel-Farid *et al.* (2020) [1] salinity stress exerts a dramatic effect on root growth by a significant reduction in root length in cucumber.

The cucumber seedlings receiving high level of proline and glucose had the longest root length because the effects of salinity on the cucumber seedlings were ameliorated and root growth was also promoted. The proline facilitated a reduction in the uptake of sodium K^+ to minimize accumulation of toxic ions in the roots and the rest of the plant leading to increased root growth. Spormann *et al.* (2023) [15] were of the same view that, proline may stimulate the root uptake of K^+ to the detriment of Na^+ by protecting the activity of K^+ transporters. Exogenous glucose enabled root respiration to occur, thus providing the carbon needed for the synthesis of carbohydrates and other molecules which contribute to increased growth of the roots.

As the concentration of combined proline and glucose increase in plants, even at the smallest concentration, the average root length of the cucumber seedlings promoted better uptake of water and nutrients and prevented toppling of the seedlings due to saline stress despite growing under salinity stress. Rady *et al.* (2016) [12] observed that all proline levels caused a significant increase in growth characteristics.

Average Root to Shoot Ratio of Cucumber Seedlings

As displayed in Tables 2; 3 and 4, the lowest average root to shoot ratio was recorded from the cucumber seedlings which did not receive proline and glucose. The cucumber seedlings were being completely affected by the salinity stress which stunted the growth of the root system. Amerian *et al.* (2024) [4] concluded that, the osmotic stress leads to nutrient imbalances, reactive oxygen species (ROS) production, and membrane damage thereby reducing yield and product quality. The osmotic stress also potentially led to ion toxicity of the saline stressed cucumbers as they would accumulate a lot of sodium and chloride ions due to the salinity of the soil. This also contributes to reduced growth of the shoots and roots.

The highest average root to shoot ratio being recorded from the cucumber seedlings receiving high level of proline and glucose indicated that the combination increased the stress tolerance of the cucumbers and also promoted the growth of the root system. Ma *et al.* (2019) [11] observed that, exogenous Glucose has the potential to alleviate salt-induced growth inhibition through regulating antioxidant capacity and nitrogen metabolism, which is associated with an improvement of cucumber growth and salt tolerance.

The significant increase (p<0.05), between all four treatments in week 4 (Table 4) suggests that the real effectiveness of the combined proline and glucose treatment on root to shoot ratio of cucumbers only shows overtime where it eventually enhances the plants' growth due to increased uptake of water and nutrients and providing structural support despite growing under salinity stress.

Average Fresh Weight & Dry Weight of Cucumber Seedlings: As indicated in Tables 2; 3 and 4, the lowest average fresh weight and dry weight was recorded from the cucumber seedlings that did not receive proline and glucose consequently the seedlings were being completely affected by the salinity stress which stunted their overall growth. The cucumber seedlings were stunted as a result of salinity stress which caused overall growth reduction, possibly due to increased water loss from the leaves owing to reverse osmosis and reduced uptake of nutrients as the roots will be experiencing cellular damage from the toxicity of Na^+ causing nutrition imbalance in the seedlings and reduced leaf area index to carry out photosynthesis effectively and efficiently. Ahmed *et al.* (2019) [2] attributes the reduction in fresh and dry weight to increased salt concentrations. Salt stress also significantly reduces the fresh and dry weight of the shoot and root (Kumar *et al.* 2021) [9].

The highest average fresh weight and dry weight recorded from the cucumber seedlings receiving high level of proline and glucose culminated in fastest overall growth because proline and glucose reduces the effects of salinity stress and promotes the overall growth of the seedlings. Exogenous proline and glucose prevented reverse osmosis consequently reducing the amount of water lost by the seedlings, keeping their cells turgid. Proline helps to reduce ion toxicity and improving antioxidant activity, and also promote the overall growth of the seedlings by increasing the metabolic and enzyme activities in processes that contribute to the growth of the plants such as in photosynthesis. Proline is also reported to contribute to photosynthesis improvement by protecting RuBisCo activity and mitochondrial electron transport chain complex II (Solomon *et al.*, 1994) [15].

The significant differences (p<0.05) on average fresh weight between all four treatments in all, suggests that as the concentration of combined proline and glucose increase, the average fresh weight of the cucumber seedlings exhibits increased biomass indicating the good health of the plants despite growing under salinity stress.

Average Leaf Length and Leaf Width of Cucumber Seedlings

The linear increase in leaf length & leaf width shows the effectiveness of proline and glucose in amending the salty stress effects. Leaf growth was promoted by proline and glucose which reduced the effects of salinity and promoted the fastest growth of the leaves. Glucose helped to increase dry matter accumulation.

The slowest leaf growth of the cucumber seedlings receiving treatment A was because of the salinity stress. Accumulation of toxic ions in the leaves results in loss of water through stomata, resulting in their cells being flaccid, thus reduction in the leaf length and leaf width. Salinity also produced signs of wilting of leaves as well as stunted growth of leaves in terms of length and width. Jenkins (2022) [7] observed that, toxic amounts of Na^+ accumulate in the leaves, inhibits photosynthesis and other processes due to stomatal closure and inhibition of gas exchange that is necessary to provide carbon for synthesis of carbohydrates.

The cucumber seedlings receiving treatment D had the fastest leaf growth because the high concentration of proline (30 mM) and glucose reduced the effects of salinity by increasing osmotic regulation, ensuring that less water is lost from the leaves via transpiration through hormonal action to close stomata. The application of exogenous glucose also helps to promote the fastest growth of the leaves due to an increase in dry matter accumulation, translating to the length and width of the leaves increase.

The significant differences ($p<0.05$) of leaf length and width, between all the four treatments suggests that as the concentration of combined proline and glucose increase, the average leaf length and leaf width of the cucumber seedlings also increases, because of the increased surface area of the leaves that improves the rates of photosynthesis and increased dry matter accumulation, consequently leading to overall growth and development of the seedlings despite growing under salinity stress. Semida *et al.* (2020) [13] is in the opinion that, exogenously-applied proline significantly enhances growth as well as photosynthetic efficiency.

Conclusion and Recommendations

The use of combined proline and glucose has a significant positive impact on the growth and development of saline stressed cucumber seedlings in areas with saline soils. As the treatment concentrations used in this study did not reach an optimum level of combined proline and glucose to use, further research is required to unravel the optimum treatment level which can be recommended to cucumber producers.

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