

Influence of Adenine Sulphate on Growth Traits of *Telfairia Occidentalis* Under Tissue Culture Conditions

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INFLUENCE OF ADENINE SULPHATE ON GROWTH TRAITS OF *TELFAIRIA OCCIDENTALIS* UNDER TISSUE CULTURE CONDITIONS

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ABSTRACT

Optimization of the protocol in tissue culture for successful micro-propagation of *Telfairia occidentalis* (fluted pumpkin) is very important. *Telfairia occidentalis* is an important leafy vegetable in West Africa, particularly noted for its nutritional and economic benefits. This study looks into how adenine sulphate (AdS) affects the growth parameters of tissue-cultured *Telfairia occidentalis* in an effort to increase the effectiveness of in-vitro propagation. In this experiment, different concentrations of AdS (0, 25, 50, 75, 100) mg/ml were added to the culture media to ascertain its effects on some key growth parameters, including shoot length, root development and leaf proliferation. The study reveals that the relationship between adenine sulphate concentration and various growth parameters in *Telfairia occidentalis* exhibits non-linear trends, with U-shaped patterns. Maximum shoot induction and leaf number was observed in the control with zero AdS concentration while leaf length gave optimal value at concentration of 100mg/ml. Root length and the number of roots exhibit a U-shaped response, with growth declining at lower concentrations (around 50 mg/ml) before increasing again at higher concentrations. Similarly, vine/shoot length increases up to 40-50 mg/ml before decreasing sharply thus showing optimal values at 50mg/ml concentration. These results suggest that specific concentrations of AdS can either promote or inhibit growth depending on the parameter of interest. This study provides detailed insights into the dose-dependent effects of AdS on the growth of *Telfairia occidentalis*. It identifies specific concentration ranges that optimize leaf, root, and shoot growth, which can be used to enhance tissue culture protocols and improve propagation outcomes for this crop.

Keywords: *Telfairia occidentalis*, tissue culture, micro-propagation, adenine sulphate.

INTRODUCTION

Telfairia occidentalis (Fluted pumpkin) is a leafy vegetable commonly cultivated in sub-saharan Africa. Its leaves and immature seeds are eaten as food. *T. occidentalis* is a vegetable with high nutritional and economic importance as it is rich in essential nutrients such as protein, iron, and vitamins (Obinaju & Asa (2015)). *T. occidentalis* is a predominant food in various African diets and possesses various health benefits, making it a subject of interest in agricultural and biotechnological research. The preservation and propagation of *T. occidentalis* faces significant challenges. It is majorly cultivated via its seeds; however, the germination capacity of the seeds decreases gradually as its humidity decreases, making them resistant to germination. The recalcitrant nature of its seed makes conservation difficult and *in vitro* techniques may be a viable option for conservation. (Akinyemi and Esuola 2012). There is a need for seeds to be preserved throughout the off-season, however, there is currently no recognized technique for managing both fruits and seeds in a way that keeps the seeds alive and vigorous. In vitro culture using plant tissue culture techniques can be the alternative for the continuous provision of plantlet for large scale production purposes (Idowu et al., 2009). Several plant species are now being propagated and mass multiplied via in vitro culture technique.

Adenine sulphate (AdS) is a growth regulator that aids enhancement of the proliferation of plantlets during in vitro propagation. It is a purine base cytokinin, known to function as a coadjuvant, promoting cell division and shoot proliferation (Sakpere et al., 2014). The capacity of adenine sulphate to promote explant growth through morphogenetic processes including callus formation and shoot induction accounts for its usefulness in tissue culture. Studies have shown that the inclusion of adenine sulphate in the culture medium significantly increases biomass accumulation in various plant species, including *T. occidentalis* (Sakpere et al., 2014; Kawshalya, 2023). Adenine sulphate has been known to function in facilitating the production of proteins and nucleic acids, both of which are essential for the development and differentiation of cells. (Mahdi, 2023). Furthermore, adenine sulphate when combined with other growth regulators, such as benzylamino purine (BAP), has been recorded to yield major results in shoot multiplication and

overall plant development (Kannan et al., 2022; Siwach & Gill, 2011). In order to derive the optimum growth outcome for *T. occidentalis* propagation in vitro, there is a need to optimize the protocol being used. The growth parameters can be greatly affected by variables like the amount of growth hormones present, the ambient temperature, and the explant source (embryo or shoot tips). For example, studies show that variable responses in shoot induction and root formation can result from altering the quantity of certain growth hormones during culturing. (Siwach & Gill, 2013; Tuan et al., 2012).

Additionally, other environmental factors such as light intensity has been shown to affect the morphological characteristics of the cultured plant, including shoot elongation and leaf morphology (Mahdi, 2023; Tuan et al., 2012). Therefore, a comprehensive understanding of these variables is essential for optimizing the tissue culture protocols for *T. occidentalis*. The role of adenine sulphate extends beyond mere growth promotion; it also influences the biochemical composition of the plant. The incorporation of adenine sulphate in the culture medium has been associated with increased levels of bioactive compounds, which are crucial for the plant's nutritional and medicinal value (Kawshalya, 2023; Falana et al., 2019). This aspect is particularly important for *T. occidentalis*, as its leaves and seeds are rich in vitamins and minerals, contributing to its health benefits. The enhancement of these bioactive compounds through tissue culture techniques could lead to improved nutritional profiles of the cultivated plants, thereby increasing their market value and health benefits (Auwal, 2023).

Protocol optimization for in vitro propagation of *T. occidentalis* is essential for the continuous production of the plant as planting materials, thereby bypassing the high dependence on its seeds which are seasonal in availability, and highly recalcitrant (Sussan et al., (2023)). Hence, this research which aims at improving the existing protocol for the in vitro propagation through the addition of varying concentrations of Adenine Sulphate (AdS) growth hormone is highly crucial. As research continues to explore the potential of adenine sulphate in enhancing the growth and quality of *T. occidentalis*, it is evident that this approach holds promise for improving the cultivation and utilization of this valuable plant.

AIMS AND OBJECTIVES

This study is aimed at optimizing the existing protocol for the in vitro cultivation of *Telfairia occidentalis* via tissue culture technique through the addition of varying concentrations of growth hormone Adenine Sulphate (AdS).

METHODOLOGY

2.1 Experimental Location and Research Design

The research was carried out in the tissue culture section of the biotechnology research unit, National Horticultural Research Institute, (NIHORT), Jericho-Idishin, Ibadan, Nigeria. Experiments were laid up in a completely randomized design under factorial arrangement.

2.2 Collection and Sterilization of Explant

Matured fruits of *T. occidentalis* pods were obtained from the Genetic Resource unit of the National Horticultural Research Institute, (NIHORT). The pods were maintained under laboratory conditions. The seeds were extracted and washed thoroughly under running tap water for 15

Minutes. Surface sterilisation was done under the laminar flow hood using one concentration of ethanol (70%) for 5 minutes, followed by two concentrations of NaOCl (10% and 15%) for 20 and 10 minutes respectively. The samples were further rinsed thrice with double distilled water. After the third rinse, the seeds were left in sterile distilled water for embryo extraction and inoculation.

2.3 Culture Establishment

The embryos were extracted and inoculated in test tubes containing 4.4g Murashige and Skoog's (1962) (MS) basal medium supplemented with 3% w/v (30g/l) sucrose, 100mg/L myoinositol and 0.7% w/v (7g/l) agar. The MS basal medium was supplemented with Adenine sulphate growth hormone at varying concentrations and a control medium which containing no hormone. The pH of the media was adjusted to 5.7 ± 0.1 with 1N of sodium hydroxide or 1N HCl before the addition of the 7g/L agar. Proper dissolution of agar in media was done in glass jars placed in microwave oven before dispensing into test-tubes for autoclaving. The melted media were dispensed in test tubes and autoclaved at 121°C for 15 mins.

Introduction of the embryos was done in sterile environment (in a Lamina flow transfer hood) and one embryo was incubated per culture tube. There were 24 tubes per treatment. The culture was

incubated in a culture room with temperature range from 30°C, 16-hr photoperiod and 500 LX light intensity

The hormone concentration is as follows;

- Adenine Sulphate (AdS) 25mg/litre
- Adenine Sulphate (AdS) 50 mg/litre
- Adenine Sulphate (AdS) 75 mg/litre
- Adenine Sulphate (AdS) 100 mg/litre
- Control; no plant growth hormones.

2.4 Data collection and Statistical analysis

Shoot length, root length, number of roots, number of shoots formed and stem girth per explants were recorded at 2 weeks, 3 weeks and 4 weeks of culture establishment. Results were based on average mean of five explants. The data generated was analyzed using Analysis of variance (ANOVA) while mean separation was done using Duncan Multiple Range Test (DMRT) at $p < 0.05$

RESULT



Fig 1 showing embryo culture after 2 weeks at zero adenine sulphate concentration



Fig 2 showing plantlet culture at 4 weeks



Fig 3 showing A: 25mg/ml conc at 4 weeks growth B: 50mg/ml conc. at 4 weeks growth C: 75mg/ml conc at 4 weeks growth.

Visualization of the effect of adenine sulphate on growth parameters

Number of leaves

Table 1: Linear relationship between average number of leaves and **adenine sulphate** conc

Factor Coding: Actual

Average number of leaves (Units)

● Design Points
— 95% CI Bands

X1 = B

Actual Factor

A = 3

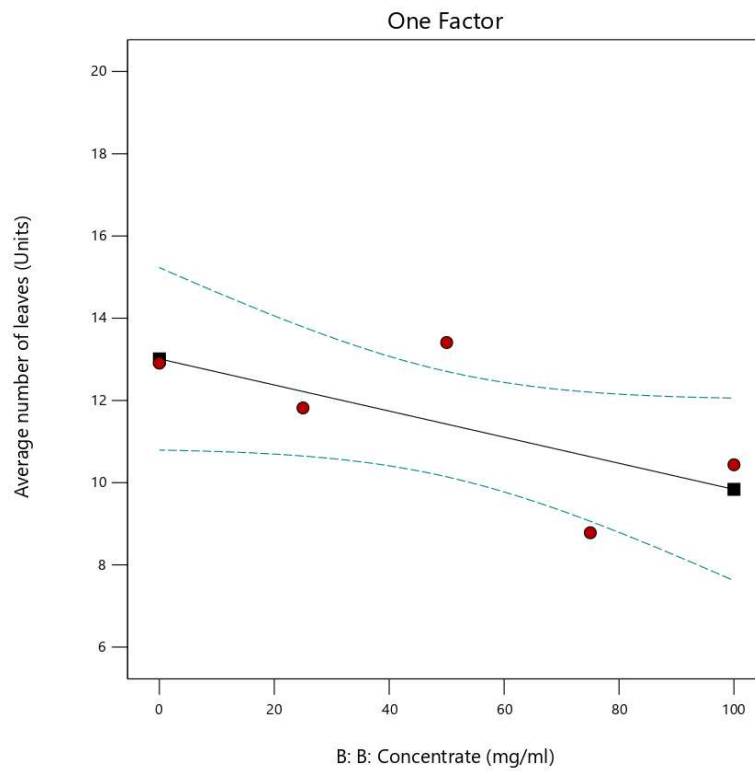


Table 2: Interaction effect of time and adenine sulphate conc. on average number of leaves

Factor Coding: Actual

Average number of leaves (Units)

Design Points:

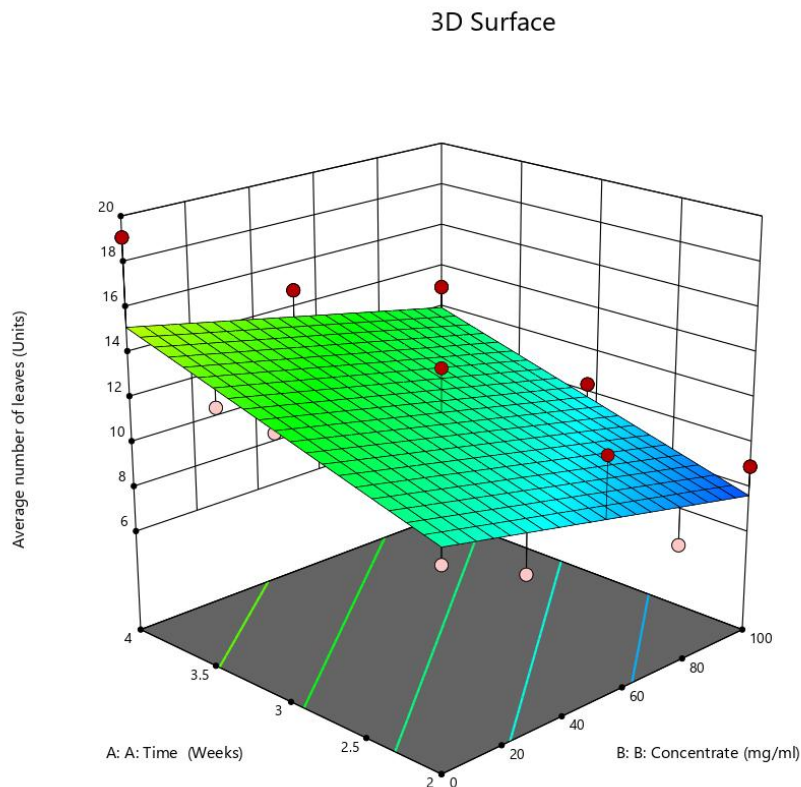
● Above Surface

○ Below Surface

6.72727 19.0909

X1 = B

X2 = A



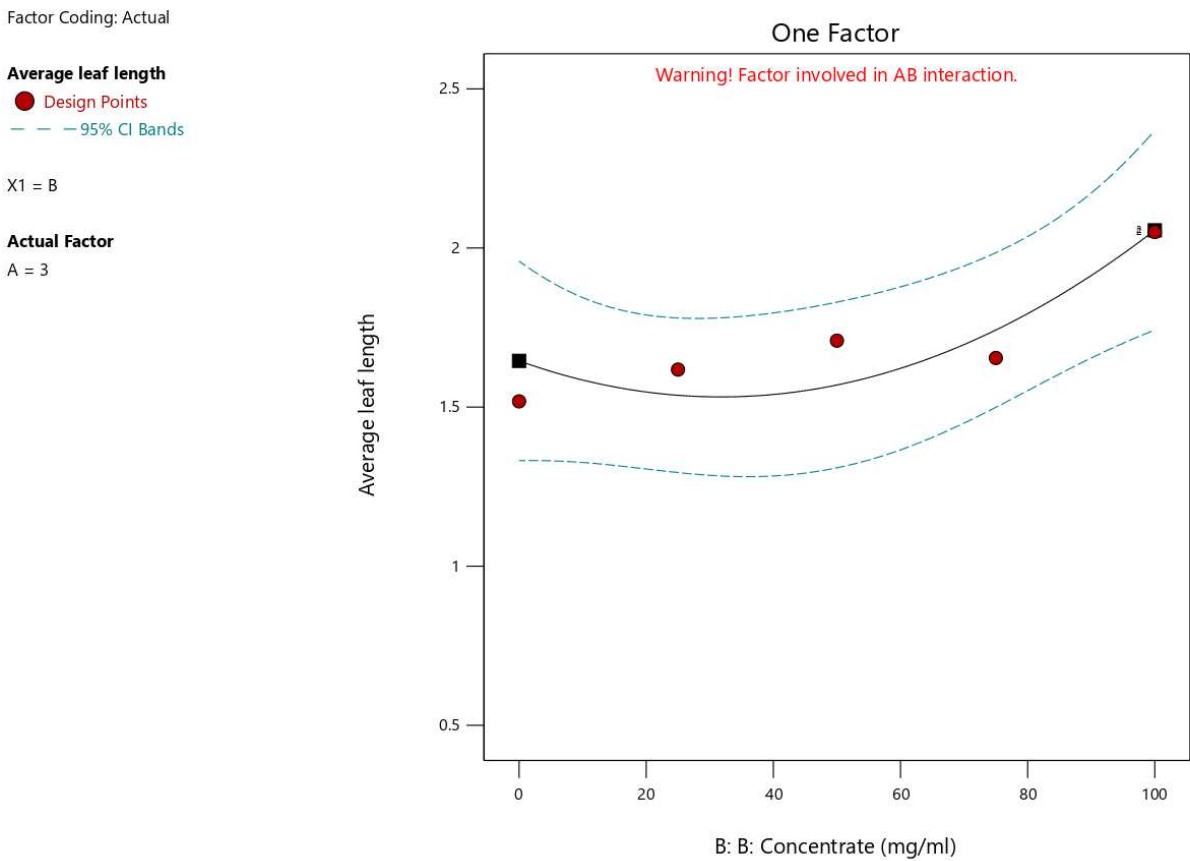
The surface is flat, indicating a linear interaction between Time and **adenine sulphate conc** on leaf production. The peak of the surface (green area) occurs at high Concentration levels and moderate to high Time periods, suggesting optimal conditions for leaf growth. The blue area at low Concentration levels and high Time periods shows the lowest average number of leaves.

As Time increases (moving along the A axis), the effect on leaf number varies depending on the Concentration level: At low Concentration levels, increasing Time leads to a decrease in leaf number. At high Concentration levels, increasing Time initially increases leaf number, then plateaus or slightly decreases.

The impact of **adenine sulphate conc** (B axis) on leaf number is more pronounced at longer Time periods: At short Time periods, changing Concentration has a relatively small effect. At longer Time periods, increasing Concentration leads to a substantial increase in leaf number. The combination of high Concentration and moderate to high Time produces the greatest number of leaves, indicating a synergistic interaction

Leaf length

Table 3: Linear relationship between **average leaf length** and **adenine sulphate conc.**



The trend line here is a slightly U-shaped, suggesting that leaf length decreases initially with increasing Concentration, then increases again at higher concentrations.

Table 4: Interaction effect between **average leaf length** and **adenine sulphate conc.**

Factor Coding: Actual

Average leaf length

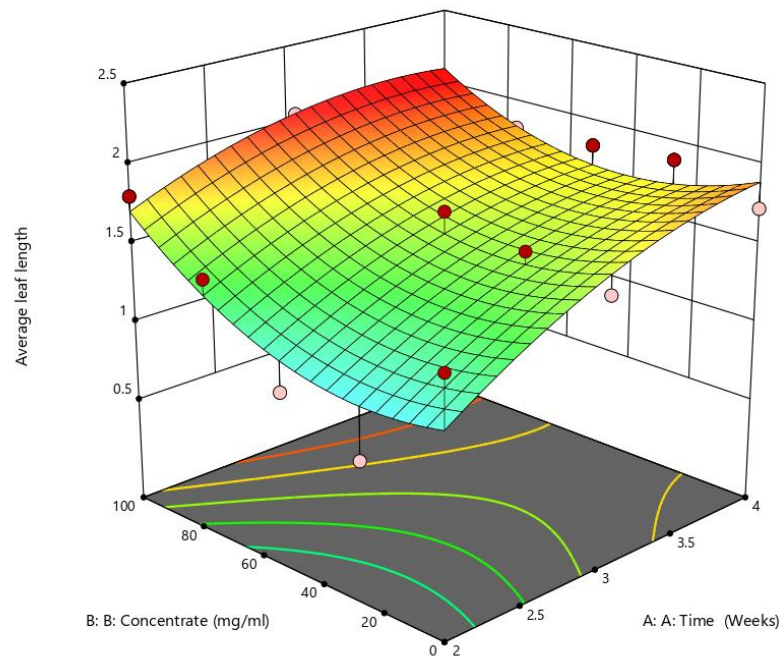
Design Points:

● Above Surface
○ Below Surface
0.709091 2.05041

X1 = A

X2 = B

3D Surface



Effect of Concentration (B: Concentration):

As the concentration increases from 0 to 100 mg/ml, there is a general trend of increasing average leaf length. This effect is more pronounced at longer time periods. The surface rises more steeply along the concentration axis, especially in the mid to high concentration range.

Effect of Time (A: Time):

The effect of time on average leaf length appears to be positive but less dramatic than that of concentration. As time progresses from 2 to 4 weeks, there is a gradual increase in leaf length, but this increase is not as steep as the one observed with increasing concentration.

Interaction Effect:

There seems to be an interaction between time and concentration. The effect of concentration on leaf length becomes more pronounced as time increases. This is evident from the steeper slope of the surface at higher time values.

Maximum Leaf Length:

The maximum average leaf length appears to occur at the highest concentration and longest time period (top right corner of the surface).

Minimum Leaf Length:

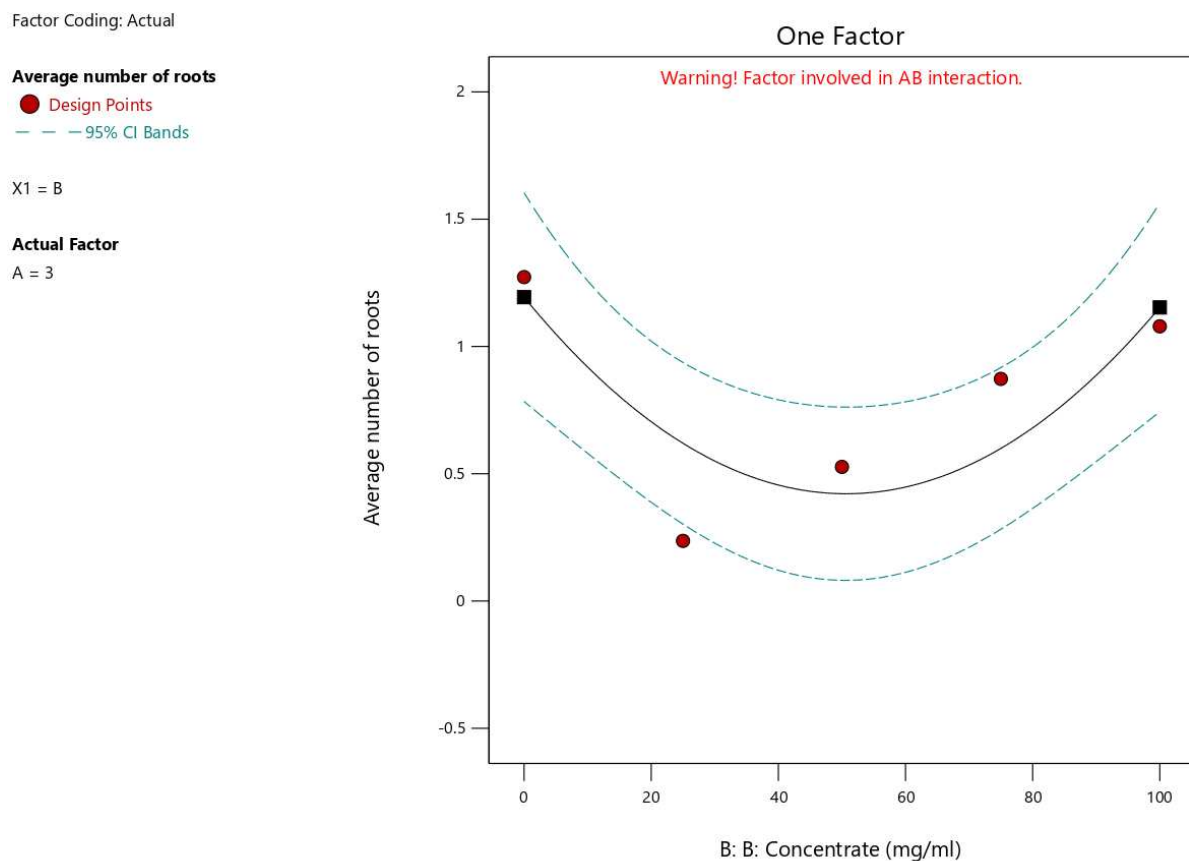
The minimum average leaf length is observed at the lowest concentration and shortest time period (bottom left corner of the surface).

Non-linearity:

The surface shows some curvature, indicating that the relationships are not perfectly linear. There's a more rapid increase in leaf length at mid to high concentrations compared to lower concentrations.

Number of Roots

Table 5: Linear relationship between concentration and average number of roots



The relationship between concentration and average number of roots is non-linear, showing a U-shaped or quadratic curve.

Initial Decrease: As concentration increases from 0 to about 50 mg/ml, there's a decrease in the average number of roots.

Minimum Point: The curve reaches its lowest point (minimum average number of roots) at approximately 50-60 mg/ml of concentration.

Subsequent Increase: After the minimum point, as concentration increases further from about 60 to 100 mg/ml, there's an increase in the average number of roots.

Range of Effect: The average number of roots varies from about 0.5 to 1.2, depending on the concentration.

Table 6: Interaction effect between concentration and average number of roots

Factor Coding: Actual

Average number of roots

Design Points:

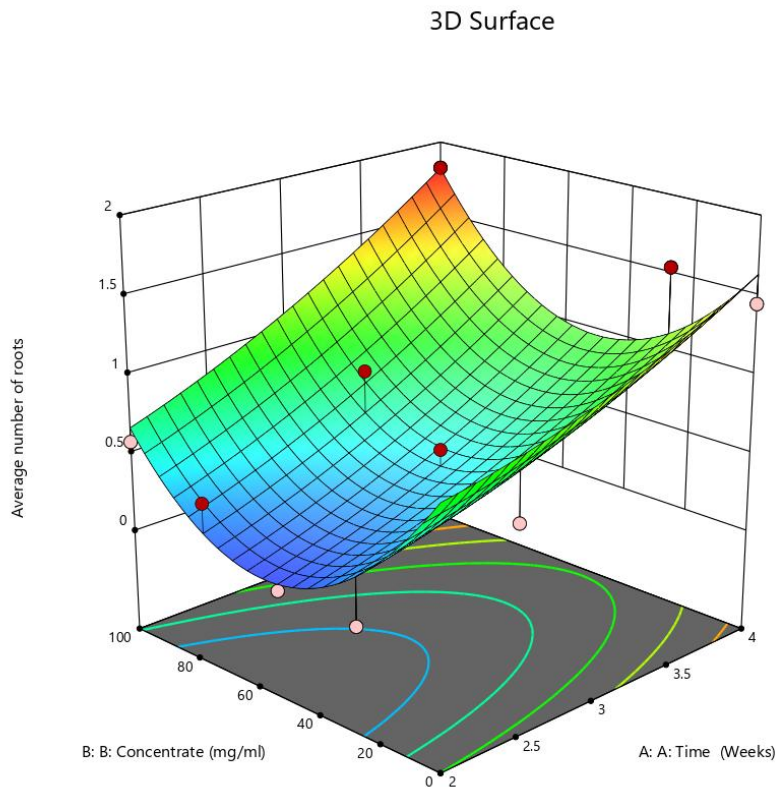
● Above Surface

○ Below Surface

0 1.82645

X1 = A

X2 = B



The surface is clearly non-planar, indicating a complex, non-linear relationship between the variables.

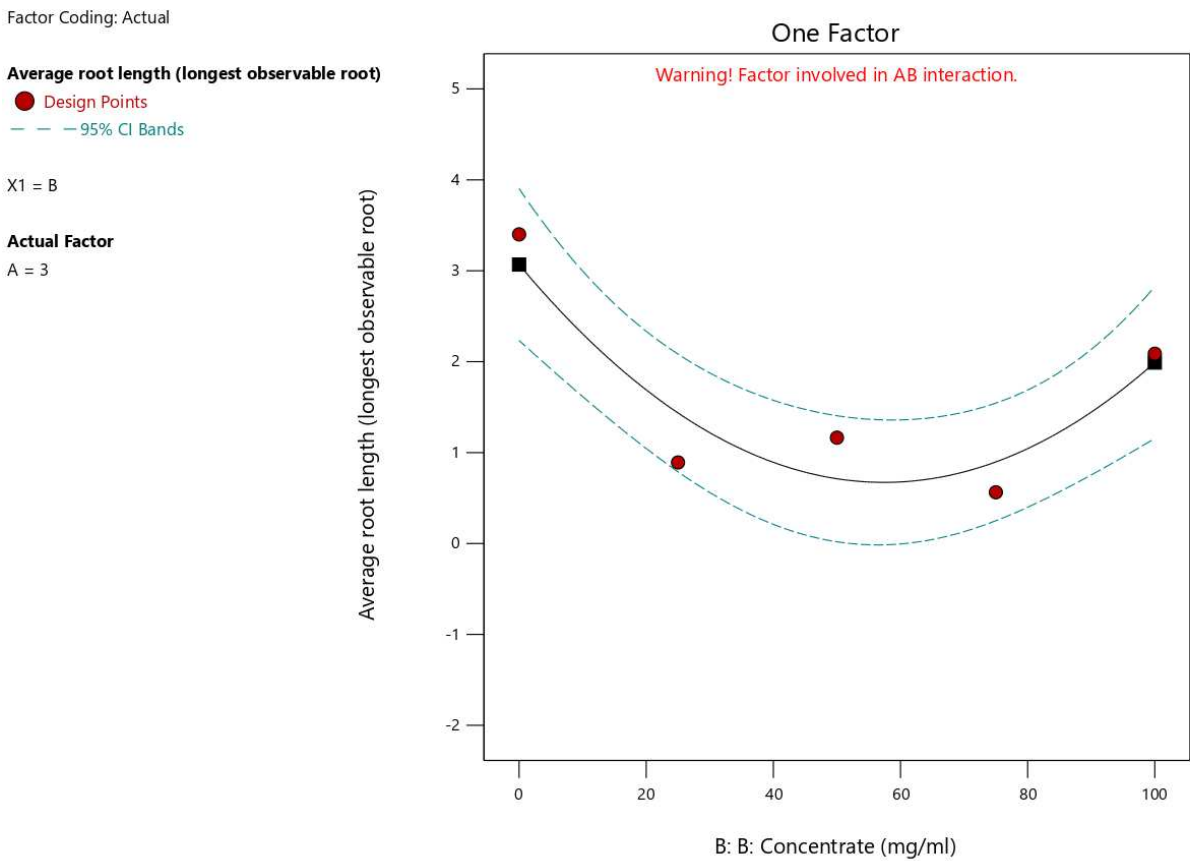
At low time values, increasing concentration initially decreases root number, then increases it at higher concentrations. This creates a U-shaped curve along the concentration axis, especially visible at lower time values.

Generally, as time increases, the average number of roots increases. This effect is more pronounced at both low and high concentrations.

There's a clear interaction between time and concentration, as the shape of the surface changes across different time and concentration values. At early time points, the U-shape along the concentration axis is more pronounced. As time increases, the surface becomes more linear along the concentration axis.

The highest average number of roots occurs at high time values and either very low or very high concentrations. The peak is slightly higher at high concentrations and high time. The lowest average number of roots is observed at low time values and mid-range concentrations.

Table 7: Linear relationship between root length and adenine sulphate concentration



The curved line represents the relationship between concentration and root length. The graph shows that as the concentration increases from 0 to about 50 mg/ml, the average root length decreases. After this point, the root length begins to increase again as the concentration continues to rise to 100 mg/ml. This creates a U-shaped curve.

Table 8: Interaction effect of root length and adenine sulphate concentration

Factor Coding: Actual

Average root length (longest observable root)

Design Points:

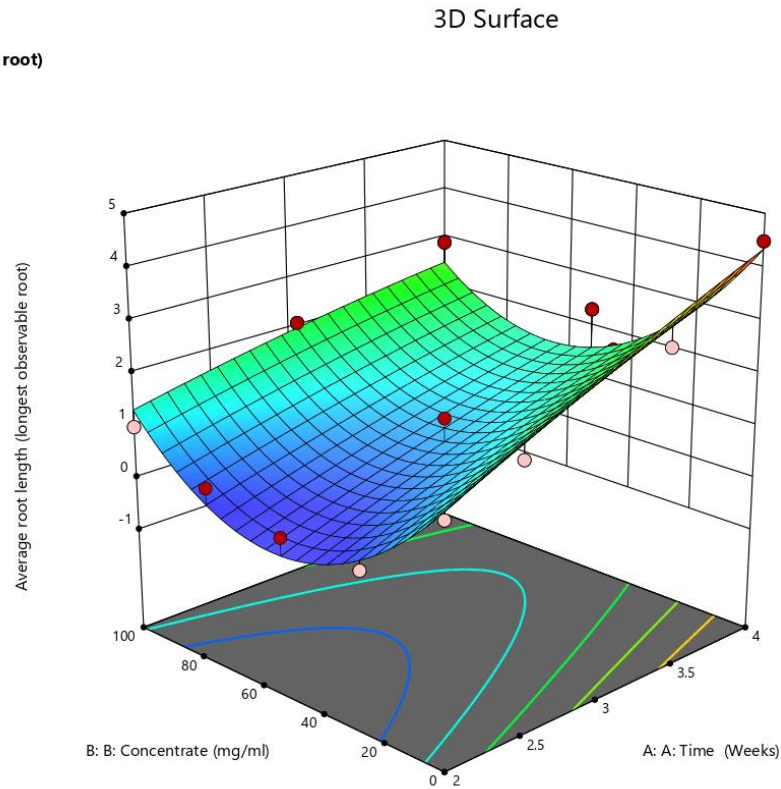
● Above Surface

○ Below Surface

0 4.49091

X1 = A

X2 = B



The curved surface indicates a complex and non-linear interaction between time and Concentration levels in affecting root length. This means the effect of one factor depends on the level of the other.

At low time values, increasing Concentration initially decreases root length, then increases it at higher concentrations. At high time values, the effect of Concentration is less pronounced, showing a more gradual curve.

At low Concentration levels, increasing time generally increases root length. At high Concentration levels, the effect of time is more complex, with an initial decrease followed by an increase in root length.

The highest root lengths appear to occur at long time and low Concentration and long time and high Concentration

Lowest root length Occurs at medium-low time and medium-high Concentration levels.

Table 9: Linear relationship between number of shoots /node and adenine sulphate conc.

Factor Coding: Actual

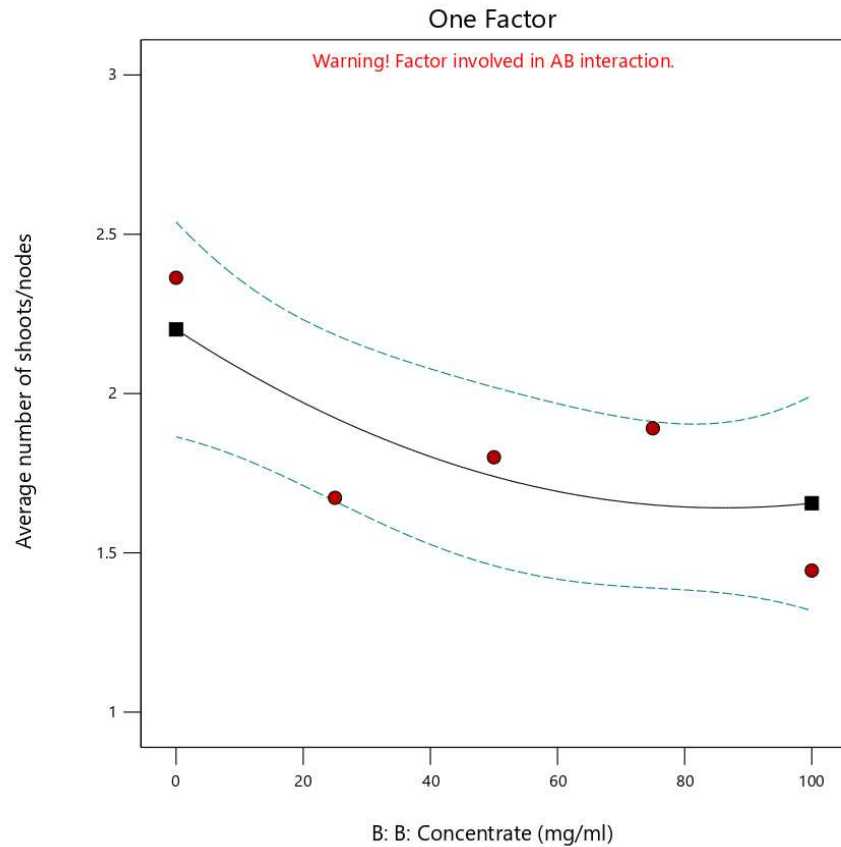
Average number of shoots/nodes

● Design Points
— 95% CI Bands

X1 = B

Actual Factor

A = 3



As the concentration of the substance (B) increases from 0 to 100 mg/ml, the average number of shoots/nodes decreases. This indicates a negative relationship between the concentration and the average number of shoots/nodes. The decrease in the average number of shoots/nodes appears to be more pronounced at lower concentrations (from 0 to around 20 mg/ml). Beyond approximately 20 mg/ml, the rate of decrease in the average number of shoots/nodes slows down, and the trend line flattens slightly as concentration approaches 100 mg/ml. This suggests that the effect of increasing concentration on reducing the average number of shoots/nodes diminishes at higher levels.

Table 10: Interaction effect of **number of shoots /node** and **adenine sulphate conc.**

Factor Coding: Actual

3D Surface

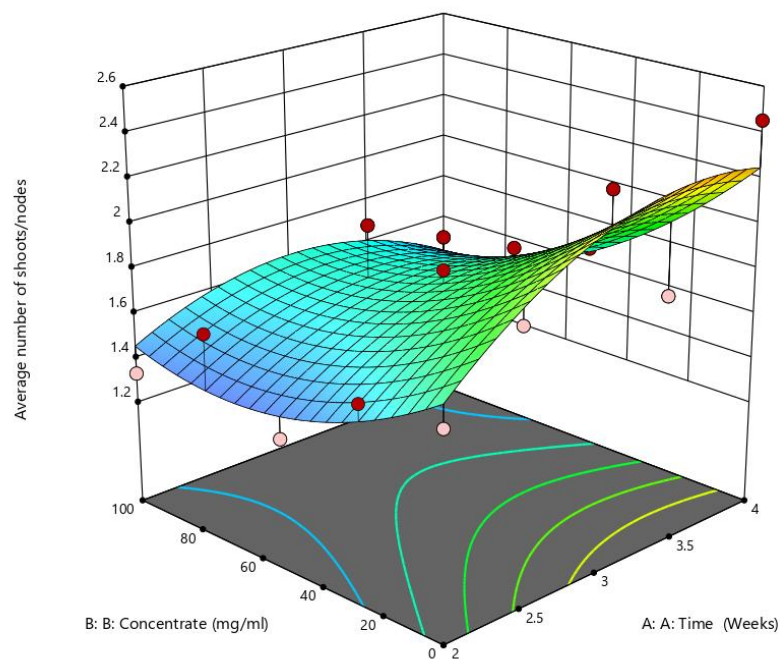
Average number of shoots/nodes

Design Points:

● Above Surface
○ Below Surface
1.30909 2.45455

X1 = A

X2 = B



At lower concentrations (closer to 0 mg/ml), the average number of shoots/nodes increases with time, reaching a peak around 3 weeks. After this peak, it appears to slightly decrease or stabilize by week 4. At higher concentrations (closer to 100 mg/ml), the effect of time on the average number of shoots/nodes is less pronounced, and the response tends to stay lower across all weeks, showing minimal growth or a decline.

At shorter time intervals (around 2 weeks), the average number of shoots/nodes decreases as concentration increases. This decline is steep initially and continues to lower levels as concentration increases to 100 mg/ml. At longer time intervals (closer to 4 weeks), the average number of shoots/nodes still tends to decrease with increasing concentration, but the response is more stable and slightly higher compared to shorter time periods.

The combined effect of time and concentration creates a curved surface, showing that lower concentrations and longer time periods favor higher average numbers of shoots/nodes. Conversely, higher concentrations and shorter time periods lead to a lower response. This surface indicates that there is a significant interaction effect between time and concentration, with the most favorable conditions for the response (higher average number of shoots/nodes) occurring at lower concentrations and intermediate time periods

Table 11: Linear relationship between vine/shoot length and adenine sulphate conc.

Factor Coding: Actual

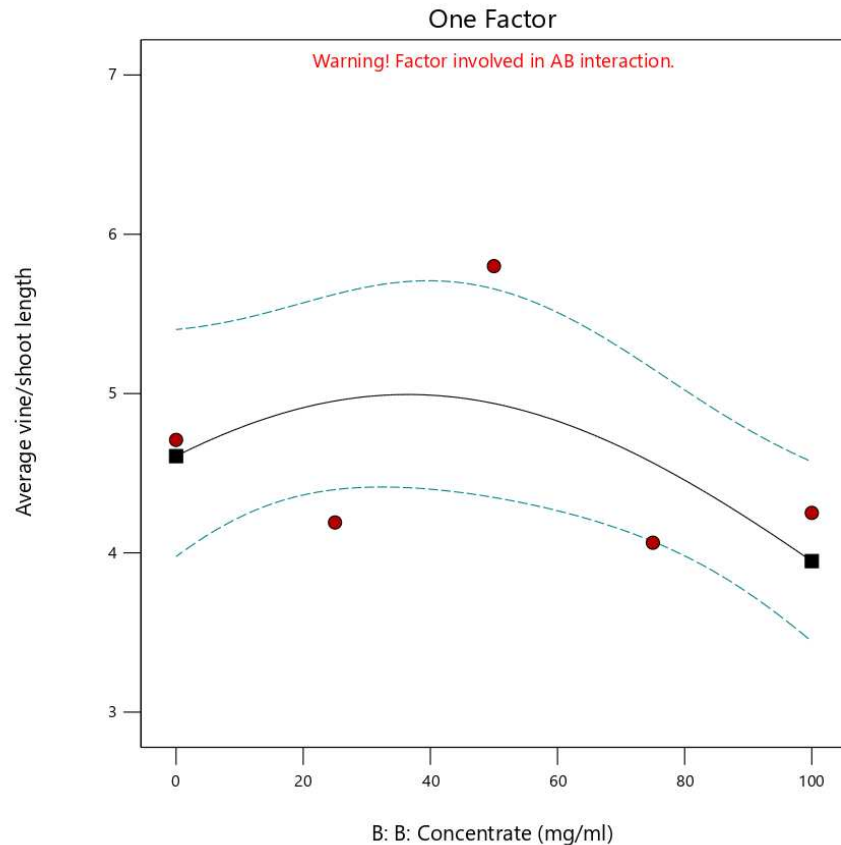
Average vine/shoot length

● Design Points
— 95% CI Bands

X1 = B

Actual Factor

A = 3



The trend of the response (average vine/shoot length) in relation to the factor B (Concentration) shows a non-linear, quadratic relationship. Initially, as the Concentration increases from 0 to about 40 mg/ml, there's a slight increase in the average vine/shoot length. The curve rises gently to a peak. Around 40-50 mg/ml Concentration, the average vine/shoot length reaches its maximum. After this peak, as the concentration continues to increase from about 50 to 100 mg/ml, there's a more pronounced decrease in the average vine/shoot length. The curve drops more steeply. This pattern suggests an optimal concentration range for maximizing vine/shoot length, beyond which higher concentrations appear to have an inhibitory effect.

Table 12: Interaction effect of vine/shoot length and adenine sulphate conc.

Factor Coding: Actual

3D Surface

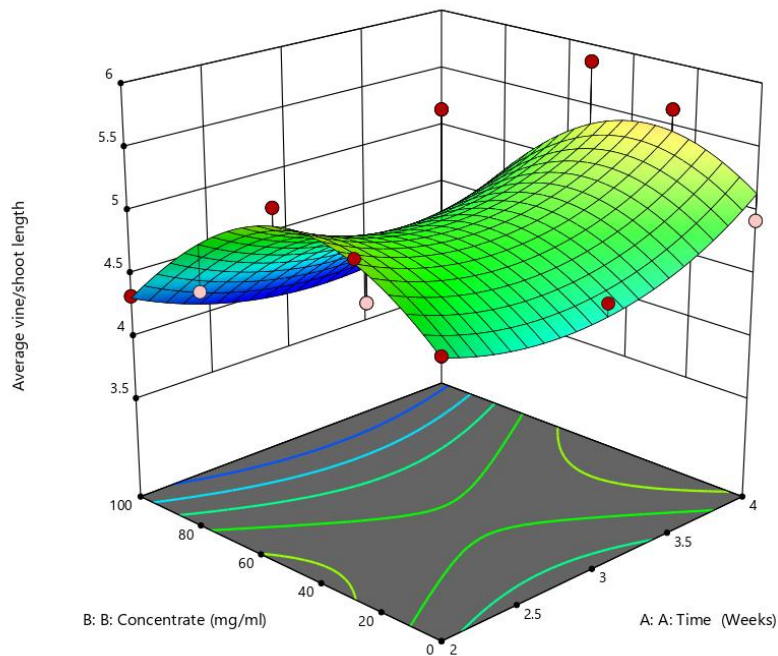
Average vine/shoot length

Design Points:

● Above Surface
○ Below Surface
4.04793 5.86364

X1 = A

X2 = B



The surface is clearly non-planar, indicating a complex, non-linear relationship between the factors and the response. The shape of the surface suggests a strong interaction between Time and Concentration. The effect of one factor depends on the level of the other. There appears to be a "peak" or optimal region where the average vine/shoot length is maximized, occurring at intermediate levels of both Time and Concentration. At low Concentration levels, increasing Time initially decreases vine/shoot length, then increases it. At high Concentration levels, the effect of Time is less pronounced. The effect of Concentration varies with Time. At early Time points, increasing Concentration generally increases vine/shoot length. At later Time points, there's a more complex relationship, with an optimal Concentration level. The color gradient and z-axis show that average vine/shoot length varies from about 4.0 to 5.8 units across the experimental range

TWO WAY ANOVA

ANOVA for Linear model

Response 1: Average number of leaves

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model: Linear	67.00	2	33.50	6.45	0.0125	significant
A-A: Time	48.11	1	48.11	9.27	0.0102	significant
B-B: Concentration	18.89	1	18.89	3.64	0.0807	Not significant
Average Leaf length						
Model: Quadratic	1.54	5	0.3072	6.85	0.0067	significant
A-A: Time	0.8881	1	0.8881	19.80	0.0016	significant
B-B: Concentration	0.3157	1	0.3157	7.04	0.0263	significant
AB	0.0503	1	0.0503	1.12	0.3170	Not significant
A ²	0.0744	1	0.0744	1.66	0.2298	Not significant
B ²	0.2073	1	0.2073	4.62	0.0601	Not significant
Average number of roots						
Model: Quadratic	3.73	5	0.7464	9.74	0.0020	significant
A-A: Time	2.15	1	2.15	28.08	0.0005	significant
B-B: Concentration	0.0031	1	0.0031	0.0402	0.8455	Not significant
AB	0.0641	1	0.0641	0.8366	0.3842	Not significant
A ²	0.0287	1	0.0287	0.3747	0.5556	Not significant
B ²	1.48	1	1.48	19.37	0.0017	significant
Average root length (longest observable root)						
Model: Quadratic	21.03	5	4.21	13.22	0.0006	significant
A-A: Time	9.40	1	9.40	29.55	0.0004	significant
B-B: Concentration	2.18	1	2.18	6.85	0.0280	significant
AB	0.7595	1	0.7595	2.39	0.1567	Not significant
A ²	0.0241	1	0.0241	0.0758	0.7893	Not significant
B ²	8.67	1	8.67	27.24	0.0005	significant
Average number of shoots/nodes						
Model: Quadratic	1.04	5	0.2076	4.00	0.0345	significant

A-A: Time	0.1557	1	0.1557	3.00	0.1173	Not significant
B-B: Concentration	0.5583	1	0.5583	10.76	0.0095	significant
AB	0.0874	1	0.0874	1.68	0.2266	Not significant
A ²	0.1430	1	0.1430	2.75	0.1314	Not significant
B ²	0.0934	1	0.0934	1.80	0.2128	Not significant
Average vine/shoot length						
Model: Quadratic	1.0069	5	0.207	2.40	0.0120	significant
A-A: Time	0.0000	1	0.0000	0.0387	0.8485	Not significant
B-B: Concentration	0.0026	1	0.0026	4.54	0.0620	Not significant
AB	0.0003	1	0.0003	0.4547	0.5171	Not significant
A ²	0.0009	1	0.0009	1.55	0.2451	Not significant
B ²	0.0031	1	0.0031	5.42	0.0449	significant

For each response variable, the table shows the results of either a linear or quadratic model. The "Model" row for each variable indicates whether the overall model is significant. In all cases here, the models are significant (p-value < 0.05).

For each factor (Time and Concentration) and their interactions, the table provides:

- Sum of Squares: Measures the variation attributed to each factor.
- df (degrees of freedom): The number of independent observations for each factor.
- Mean Square: The Sum of Squares divided by df.
- F-value: The test statistic used to determine significance.
- p-value: Indicates the statistical significance of each factor.

Interpretation of p-values:

- $p < 0.05$ is considered statistically significant.
- $p \geq 0.05$ is considered not statistically significant.

Key findings for each response variable:

- a. Average number of leaves: Only Time has a significant effect (linear model).
- b. Average leaf length: Both Time and Concentration have significant linear effects (quadratic model).
- c. Average number of roots: Time has a significant linear effect, and Concentration has a significant quadratic effect (B²).

d. Average root length: Time and Concentration have significant linear effects, and Concentration also has a significant quadratic effect (B^2).

e. Average number of shoots/nodes: Only Concentration has a significant linear effect.

f. Average vine/shoot length: Only the quadratic effect of Concentration (B^2) is significant.

6. Interactions (AB) and quadratic effects of Time (A^2) are not significant for any of the response variables.

This analysis suggests that Time and Concentration affect different aspects of plant growth in various ways, sometimes linearly and sometimes non-linearly.

Post hoc

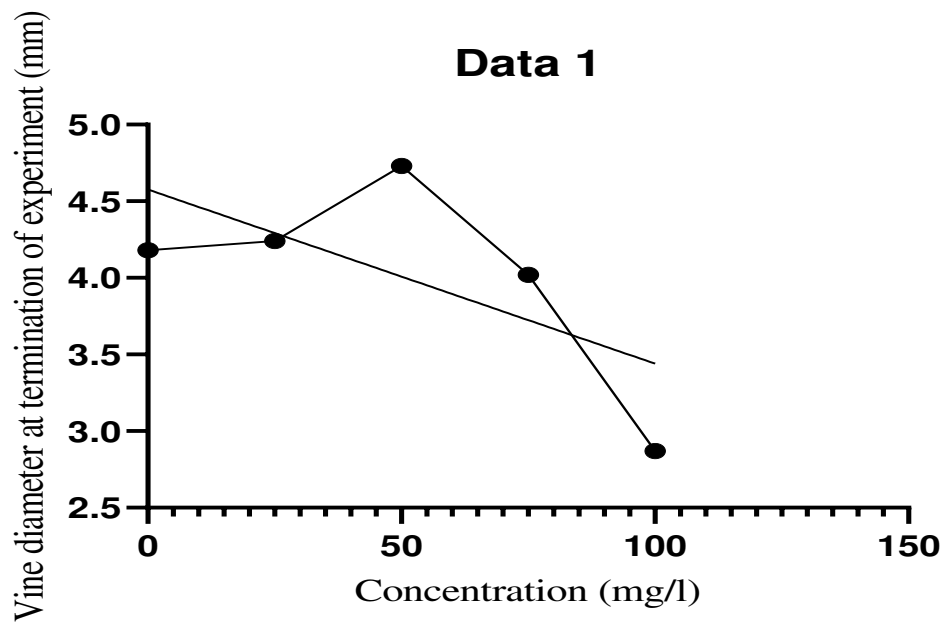
Vine_diameter_at_termination_of_experiment

	Concentration	N	Subset for alpha = 0.05	
			1	2
Duncan ^{a,b}	75.00	13	2.9056b	
	100.00	11		4.1196a
	50.00	12		4.2364a
	25.00	13		4.2577a
	.00	12		4.7255a
	Sig.		1.000	.089

Vine_diameter_at_termination_of_experiment

	Concentration	N	Subset for alpha = 0.05	
			1	2
Duncan ^{a,b}	75.00	13	2.9056b	
	100.00	11		4.1196a
	50.00	12		4.2364a
	25.00	13		4.2577a
	.00	12		4.7255a
	Sig.		1.000	.089

Means Plots



Concentration	Vine diameter at termination of experiment	SD
0	4.18 ^a	.91059

25	4.24 ^a	.95277
50	4.73 ^a	.65039
75	4.02 ^a	.87022
100	2.87 ^b	.47700

The Duncan test was used for multiple comparisons, with an alpha level of 0.05.

The test identified two distinct subsets:

Subset 1: Contains only the 75.00 mg/ml concentration

Subset 2: Contains 0.00, 25.00, 50.00, and 100.00 mg/ml concentrations

Mean vine diameters:

0.00 mg/ml: 4.7255 mm

25.00 mg/ml: 4.2577 mm

50.00 mg/ml: 4.2364 mm

75.00 mg/ml: 2.9056 mm

100.00 mg/ml: 4.1196 mm

The 75.00 mg/ml concentration resulted in significantly smaller vine diameters (2.9056 mm) compared to all other concentrations. This is denoted by the "b" superscript.

There were no statistically significant differences among the vine diameters for 0.00, 25.00, 50.00, and 100.00 mg/ml concentrations. These are all marked with an "a" superscript.

The control (0.00 mg/ml) had the largest mean diameter, though not significantly different from 25.00, 50.00, and 100.00 mg/ml concentrations.

There appears to be a non-linear relationship between concentrate level and vine diameter. The diameter decreases as concentration increases up to 75 mg/ml, but then increases again at 100 mg/ml.

The significance value of 0.089 for the second subset indicates that while these means are not significantly different from each other, there might be a trend or weak evidence of some differences. This analysis suggests that a concentration of 75 mg/ml has a unique and significant inhibitory effect on vine diameter growth compared to other concentrations, including both lower and higher levels. The other concentrations, including the control, produce similar results in terms of vine diameter.

DISCUSSION AND CONCLUSION

Number of Leaves

The analysis shows a significant linear relationship between the average number of leaves and adenine sulfate concentration, with a notable decrease in leaf number as concentration increases. This finding aligns with previous studies that suggest high concentrations of growth regulators can inhibit leaf production due to potential toxicity or nutrient imbalances (Mohamed & Ibrahim, 2011; Kumar et al., 2014). The interaction effect between time and concentration indicates that while higher concentrations can promote leaf growth initially, prolonged exposure leads to a decline in leaf numbers, suggesting a threshold beyond which the benefits of adenine sulfate diminish (Jaiswal et al., 2021; Kadam et al., 2016).

Leaf Length

The average leaf length exhibits a non-linear response to adenine sulfate concentration, initially decreasing at lower concentrations before increasing at higher levels. This relationship indicates that moderate concentrations may be optimal for leaf elongation, corroborating findings from other studies that highlight the importance of finding a balance in growth regulator concentrations for optimal plant development (Oraon & Mondal, 2021; Escudero-Almanza et al., 2022). The positive effect of time on leaf length is also evident, suggesting that longer exposure to adenine sulfate enhances growth, particularly at higher concentrations (Mohamed & Ibrahim, 2011; Kumar et al., 2014).

Number of Roots and Root Length

The average number of roots shows a U-shaped curve in response to adenine sulfate concentration, with a decrease observed at lower concentrations followed by an increase at higher concentrations. This pattern suggests that low concentrations may not provide sufficient stimulation for root development, while excessive concentrations could lead to root inhibition (Jaiswal et al., 2021; Samantaray et al., 2013). The interaction between time and concentration further complicates this relationship, indicating that root growth is more responsive to concentration changes at longer time intervals (Sharma et al., 2020; Stevens & Pierce, 2013).

Number of Shoots

The average number of shoots demonstrates a negative correlation with increasing adenine sulfate concentration, particularly at lower levels. This finding is consistent with the notion that excessive growth regulators can lead to reduced shoot proliferation, as observed in other plant species (El-Soda et al., 2019; Qosim et al., 2015). The interaction of time with concentration suggests that lower concentrations favor shoot development over time, while higher concentrations suppress shoot growth (Alagesaboopathi, 2016; Barroso et al., 2017).

Vine/Shoot Length

The vine or shoot length follows a non-linear relationship with adenine sulfate concentration, peaking at moderate levels before declining at higher concentrations. This pattern indicates the presence of an optimal concentration range for maximizing shoot length, which is critical for

overall plant vigor (Bahamonde et al., 2023; Thakur et al., 2011). The interaction between time and concentration further emphasizes the need for careful management of growth conditions to achieve desired growth outcomes (Kadam et al., 2016; Al-Sulaiman, 2010).

Conclusion

In summary, the effects of adenine sulfate on plant growth parameters are multifaceted, with concentration and time playing crucial roles in determining outcomes. The findings suggest that while adenine sulfate can enhance certain growth metrics, excessive concentrations may lead to detrimental effects. Therefore, it is essential to optimize both concentration and exposure duration to achieve the best growth results. Future research should focus on elucidating the underlying mechanisms of these interactions to refine application strategies in plant cultivation.

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