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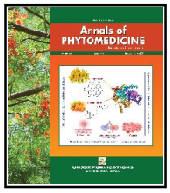

Original Article : Open Access

Influence of plant growth regulators and sucrose on macronutrient, biochemical composition and phytochemical content of *Andrographis echioides* (L.) Nees. suspension cultures

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Abstract

The study examines the combined influence of plant growth regulators (PGRs) and varying sucrose concentrations on growth, nutrient content, biochemical composition, and andrographolide accumulation in suspension cell cultures of *Andrographis echioides* (L.) Nees., a medicinal plant valued for its therapeutic properties. Suspension cultures were initiated in MS liquid medium supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), naphthaleneacetic acid (NAA), and 6-benzylaminopurine (BAP), along with sucrose levels ranging from 1% to 4%. Growth parameters such as packed cell volume (PCV), cell wet weight, dry weight, macronutrient content (nitrogen, phosphorus, and potassium), biochemical components (protein, phenol, and carbohydrates) and andrographolide content, were assessed at intervals of 15, 30, 45, and 60 days. Optimal results were obtained with the treatment combination MS + 2 mg/l 2,4-D + 2 mg/l NAA + 2 mg/l BAP + 3% sucrose (T8), which significantly enhanced biomass, nutrient, and biochemical accumulation by day 45, while andrographolide content peaked at day 60. These results highlight the critical role of a well-balanced hormonal combination and an appropriate carbon source for improving nutrient uptake and biomass yield in suspension cultures. The optimized culture conditions established through this study offer a reliable and effective platform for mass-producing of bioactive metabolites from *A. echioides* under controlled *in vitro* environments.

1. Introduction

Andrographis echioides (L.) Nees., classified under the family Acanthaceae, is a medicinal herb native to South India and has long been used in various indigenous medical systems. It is well known for its wide range of therapeutic properties, including antibacterial (Maheshwari *et al.,* 2021), hepatoprotective (Basu *et al.,* 2009a), anti-inflammatory, anti-ulcer (Raja and Jeevanreddy, 2014), analgesic, and antipyretic activities (Basu *et al.,* 2009b). Despite its notable pharmacological value, the quality and yield of bioactive metabolites obtained from wild and field-grown *A. echioides* are often inconsistent. Factors such as environmental fluctuations, pest infestations, disease incidence, and the use of agrochemicals contribute to variations in phytochemical profiles and compromise the overall quality of the harvested plant material. To address these challenges, *in vitro* plant culture especially cell suspension culture technique offers a controlled and sustainable alternative for producing uniform, high-quality phytochemical compounds with improved consistency and reliability.

Quantitative analysis of secondary metabolites, including andrographolide-a key diterpene lactone in *A. echioides* is essential for evaluating the success of *in vitro* production systems. Currently, andrographolide is incorporated into formulations for treating infections, liver disorders, and immune deficiencies, driving its demand across Asia, Europe, and North America. Its natural origin and therapeutic efficacy have further positioned it as a valuable compound in plant-based drug development (Li *et al.,* 2022).The global market for andrographolide is projected to reach USD 205.8 million by 2025, with the Asia Pacific region contributing 36.78%. Major markets include China (41.61%), India (17.97%), and South East Asia (16.64%). Driven by rising demand in herbal medicine and pharmaceuticals, the industry is expected to grow at a CAGR of 9.6%, reaching USD 157.1 million by 2033 (Andrographolide Market Report, 2025). Optimizing nutrient uptake and assimilation *in vitro* can greatly enhance the nutritional value and productivity of cultured plant tissues. Nitrogen is essential for the synthesis of amino acids, proteins, and other nitrogenous compounds (Ohyama, 2010); phosphorus supports energy transfer and nucleic acid metabolism (Tian *et al.,* 2019); and potassium helps regulate osmotic balance and activate key enzymes (Kumar *et al.,* 2020). Moreover, the biosynthetic capabilities of plant cells *invitro* are influenced by external inputs such as PGRs and carbon sources.

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Despite the known medicinal potential of *A. echioides*, comprehensive research on its physiological and biochemical responses under suspension culture conditions is still lacking. This investigation focuses on assessing how different combinations of PGRs and sucrose concentrations influence nutrient dynamics (NPK), biochemical characteristics, and andrographolide levels in suspension cultures of *A. echioides*. The findings are expected to contribute significantly to the development of efficient, scalable systems for medicinal plant biotechnology and phytopharmaceutical production.

2. Materials and Methods

The current analysis on the impact of PGRs and sucrose concentrations on the growth and nutrient content of suspension cell cultures in *A. echioides* was conducted at the Tissue Culture Laboratory, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The plant was authenticated by the Botanical Survey of India. The authenticated specimen number is BSI/SRC/5/23/2025-26/Tech./475/19.05.2025.

2.1 Plant material and callus induction

Young, healthy leaf explants of *A. echioides* (Figure 1) were collected and surface-sterilized in 0.1% HgCl, for 3 min, then rinsed thoroughly (3-5 times) with sterile deionized water. The sterilized leaf bits were inoculated onto MS basal medium supplemented with varying concentrations of 2,4-D (2.0 mg/l) + NAA (2.0 mg/l) + BAP (2.0 mg/l) to stimulate callus formation. The cultures were maintained under controlled environmental conditions for 21 days at 25 ± 2°C under a 16 h photoperiod supplied by cool-white fluorescent lamps for obtaining profuse callus growth.

Figure 1: *A. echioides* mother plant.

2.2 Establishment of suspension cultures

Actively growing friable calli were transferred to 100 ml of MS liquid medium supplemented with the respective concentrations of PGRs in two experimental sets, as described in Table 1.

Set I (growth regulator experiment)

MS medium containing 2,4-D, NAA, and BAP at concentrations ranging from 1.0 to 2.5 mg/l, both individually and in different combinations.

Set II (sucrose concentration experiment)

A fixed concentration of 2.0 mg/l of each growth regulator was combined with varying sucrose levels (1%, 2%, 3%, and 4%).

2.3 Growth assessment

Growth was assessed at four-time intervals: 15, 30, 45, and 60 days after inoculation. The following growth parameters were recorded:

Packed cell volume (PCV%)

Measured by allowing cells to settle in a graduated centrifuge tube and recording the volume occupied by the cells.

Cell wet weight (g/l)

The cultures were filtered using Whatman No. 1 filter paper, and the collected biomass was immediately weighed to determine the fresh weight per liter of culture medium.

Cell dry weight (g/l)

The filtered biomass was oven-dried at 60°C up to a constant weight was obtained the dry weight per liter of culture medium was then recorded.

The cultures were maintained under controlled environmental settings.

Table 1: Details of different treatments with PGR combinations and sucrose level combinations

Treatment	PGR combinations	Treatment	Sucrose concentrations
T_1	MS basal	T_1	MS basal
T_2	MS + 2, 4-D 1 mg/l + BAP 1 mg/l	T_2	MS + 2, 4-D 2 mg/l+ NAA 2 mg/l + 1% sucrose
T_3	MS + 2, 4-D 1.5 mg/l + BAP 1.5 mg/l	T_3	MS + 2, 4-D 2 mg/l + NAA 2 mg/l + 2% sucrose
T_4	MS + 2, 4-D 2 mg/l + BAP 2 mg/l	T_4	MS + 2, 4-D 2 mg/l + NAA 2 mg/l + 3% sucrose
T_5	MS + 2, 4-D 2.5 mg/l + BAP 2.5 mg/l	T_5	MS + 2, 4-D 2 mg/l + NAA 2 mg/l + 4% sucrose
T_6	MS + 2, 4-D 1 mg/l + BAP 1 mg/l + NAA 1 mg/l	T_6	MS + 2, 4-D 2 mg/l+ NAA 2 mg/l + BAP 2 mg/l + 1% sucrose
T_7	MS + 2, 4-D 1.5 mg/l + BAP 1.5 mg/l + NAA 1.5 mg/l	T_7	MS + 2, 4-D 2 mg/l + NAA 2 mg/l + BAP 2 mg/l + 2% sucrose
T_8	MS + 2, 4-D 2 mg/l + BAP 2 mg/l + NAA 2 mg/l	T_8	MS + 2, 4-D 2 mg/l + NAA 2 mg/l + BAP 2 mg/l + 3% sucrose
T_9	MS + 2, 4-D 2.5 mg/l + BAP 2.5 mg/l + NAA 2.5 mg/l	T_9	MS + 2, 4-D 2 mg/l + NAA 2 mg/l + BAP 2 mg/l + 4% sucrose

2.4 Estimation of nutrient content (NPK) and biochemical studies

Nitrogen was determined by Kjeldahl digestion, phosphorus by the vanadomolybdate yellow colorimetric assay, and potassium by flame photometry, soluble protein by Bradford method (1976), carbohydrates by Hedge and Hofreiter (1962) method, and phenol content by Harborne (1973) method. Growth and nutrient parameters were compared across different treatment and days combinations (T × D interaction).

2.5 Estimation of andrographolide

The andrographolide content in the samples was determined from the HPLC chromatogram using the following formula and expressed in percentage (Kumaran *et al*., 2003).

$$\text{Andrographolide content (\%)} = \frac{\text{Area of the standard}}{\text{Area of the sample}} \times \frac{\text{Weight of the standard}}{\text{Weight of the sample}} \times \frac{\text{Volume injected (standard)}}{\text{Volume injected (sample)}} \times \text{Volume made up}$$

2.6 Data analysis

Data analysis was conducted using analysis of variance (ANOVA) to assess the statistical significance of the results.

3. Results

3.1 Influence of PGRs on growth parameters

The PCV values increased steadily with culture duration, reaching their peak on the 45th day before declining. Treatment T_8 recorded the highest PCV at 45th days (6.14%), followed closely by T_4 (6.03%), which included 2.0 mg/l of 2,4-D and NAA without BAP (Table 2). The decline in PCV beyond 45th days indicates a shift toward the stationary phase of cell growth, likely due to nutrient limitation or accumulation of toxic metabolites. The biomass accumulation, measured as cell wet and dry weights, followed a trend similar to PCV. The highest cell wet weight (40.56 g/l) and dry weight (11.38 g/l) were observed in T_8 at 45th days (Table 3). T_4 and T_3 also supported substantial biomass accumulation, though consistently lower than T_8.

Table 2: Influence of PGRs on packed cell volume (%) in *A. echioides*

Treatments	PCV (%) days				T-mean
	15	30	45	60	
T_1	0.46	0.60	0.66	0.25	0.49
T_2	0.60	1.50	2.18	1.12	1.35
T_3	1.01	2.76	5.05	3.81	3.16
T_4	1.30	3.03	6.03	4.29	3.66
T_5	0.70	1.66	3.94	2.76	2.26
T_6	0.52	1.13	1.84	0.73	1.05
T_7	0.78	1.20	1.65	0.84	1.12
T_8	1.12	3.13	6.14	5.12	3.88
T_9	0.61	1.41	3.29	2.13	1.86
D-mean	0.79	1.82	3.42	2.34	2.09

Interaction effect	SE(d)	CD (0.05)
T	0.022	0.043
D	0.014	0.029
TD	0.043	0.086

*T-treatments, D-days.

3.1.1 Comparative growth trends

Overall, T_8 significantly outperformed other treatments, indicating that a balanced combination of auxins (2,4-D and NAA) and cytokinins (BAP) at optimal concentrations enhances cell division and biomass production. Treatments with higher concentrations of PGRs (T_9) or with only auxins ($T_2 - T_5$) showed relatively lower efficiency, highlighting the importance of hormonal synergy and balance for optimal culture performance.

Table 3: Influence of PGRs on cell wet weight and cell dry weight (g/l) in *A. echioides*

Treatments	Cell wet weight (g/l) days				T-mean	Cell dry weight (g/l) days				T-mean
	15	30	45	60		15	30	45	60	
T_1	4.05	5.20	5.85	3.07	4.54	1.07	1.23	1.48	0.72	1.13
T_2	4.65	6.67	13.26	9.14	8.43	1.18	1.44	1.68	1.08	1.34
T_3	11.25	17.05	35.73	29.40	23.36	2.91	3.84	8.80	7.34	5.72
T_4	10.31	17.15	37.48	30.22	23.79	3.10	4.73	10.32	9.36	6.88
T_5	7.76	9.43	18.17	12.27	11.91	1.28	1.80	2.08	1.80	1.74
T_6	9.66	11.91	25.30	18.21	16.27	2.48	2.95	3.51	2.69	2.91
T_7	6.77	12.70	28.24	20.22	16.98	2.72	3.43	6.37	5.67	4.55
T_8	13.03	20.28	40.56	33.49	26.84	3.95	5.67	11.38	9.95	7.74
T_9	9.52	10.38	24.04	15.55	14.87	1.65	2.32	2.46	2.03	2.11
D-mean	8.56	12.31	25.40	19.06	0.403	2.26	3.04	5.34	4.51	3.79

Interaction effect	Cell wet weight (g/l)		Cell dry weight (g/l)	
	SE(d)	CD (0.05)	SE(d)	CD (0.05)
T	0.383	0.763	0.118	0.235
D	0.255	0.509	0.078	0.156
TD	0.766	1.526	0.235	0.469

*T-treatments, D-days.

3.2 Influence of sucrose concentrations on growth parameters

Sucrose concentration significantly influenced the growth and biomass accumulation of *A. echioides* suspension cultures (Figure 2). Among the tested concentrations, 3% sucrose (T_8) was found to be optimal, yielding the highest packed cell volume of 6.14% on the 45th day of culture. When the sucrose concentration was increased beyond 3% to 4%, a decline in PCV was noted, indicating potential osmotic stress that may have hindered cell proliferation. Similarly, the treatment T_8 resulted in the highest cell wet weight (40.56 g/l) and dry weight (11.38 g/l). Lower sucrose levels (1–2%) supported moderate growth, whereas the 4% level led to reduced biomass, likely due to disrupted osmotic balance and impaired metabolic activity (Cui *et al*., 2010). The outcomes were shown in Table 4 and 5.

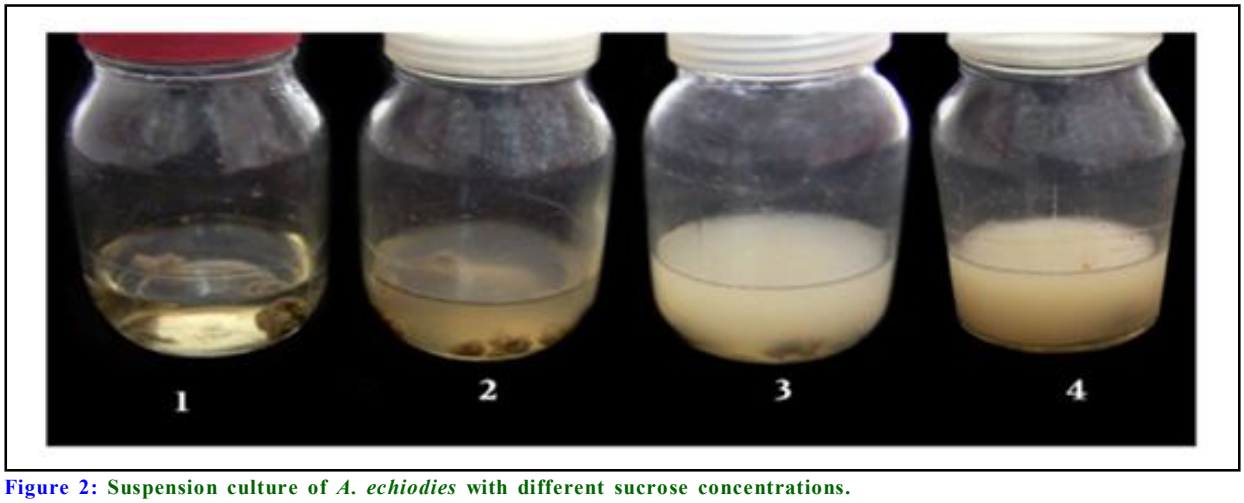

Figure 2: Suspension culture of *A. echiodies* with different sucrose concentrations.

Table 4: Influence of sucrose concentrations on packed cell volume (%) in *A. echioides*

Treatments	PCV (%) days				T-mean
	15	30	45	60	
T_1	0.45	0.57	0.50	0.20	0.43
T_2	0.56	1.12	2.47	1.32	1.37
T_3	1.08	2.06	4.25	3.05	2.61
T_4	1.30	3.03	6.03	4.29	3.66
T_5	0.77	1.23	3.11	1.95	1.76
T_6	0.62	1.05	2.35	1.54	1.39
T_7	1.16	2.45	5.09	3.22	2.98
T_8	1.12	3.13	6.14	5.12	3.88
T_9	0.86	1.52	3.69	2.48	2.14
D-mean	0.88	1.79	3.74	2.57	2.25

Interaction effect	SE(d)	CD (0.05)
T	0.058	0.115
D	0.038	0.077
TD	0.115	0.230

*T-treatments, D-days.

Table 5: Influence of sucrose concentrations on cell wet weight and cell dry weight (g/l) in *A.echioides*

Treatments	Cell wet weight (g/l) days				T-mean	Cell dry weight (g/l) days				T-mean
	15	30	45	60		15	30	45	60	
T_1	3.98	4.81	6.00	3.02	4.54	0.81	1.03	0.91	0.68	0.86
T_2	4.55	8.63	10.92	7.61	8.43	1.29	1.55	3.12	1.90	1.96
T_3	9.65	13.45	34.88	28.42	23.36	2.87	4.03	9.25	7.90	6.01
T_4	10.31	17.15	37.48	30.22	23.79	3.10	4.73	10.32	9.36	6.88
T_5	5.58	11.17	22.50	17.50	11.91	2.10	2.93	7.22	5.80	4.51
T_6	5.02	9.75	24.95	19.50	16.27	1.71	2.08	5.08	3.92	3.20
T_7	7.76	12.82	32.10	21.55	16.98	3.38	4.91	10.33	8.75	6.84
T_8	13.03	20.28	40.56	33.49	26.84	3.95	5.67	11.38	9.95	7.74
T_9	5.40	18.17	20.00	9.05	14.87	2.49	3.17	8.25	6.92	5.21
D-Mean	7.25	12.91	25.49	18.93	16.15	2.41	3.35	7.32	6.13	4.80

Interaction effect	Cell wet weight (g/l)		Cell dry weight (g/l)	
	SE(d)	CD (0.05)	SE(d)	CD (0.05)
T	0.247	0.492	0.116	0.232
D	0.165	0.328	0.078	0.155
TD	0.494	0.984	0.233	0.464

*T-treatments, D-days.

3.3 Influence of PGRs on nutrient content and biochemical components

The application of PGRs significantly influenced nitrogen, phosphorus, and potassium (NPK) content in *A. echioides* suspension cultures.

3.3.1 NPK content

Maximum nitrogen (0.578%), phosphorus (0.217%) and potassium (0.357%) were observed in T_8 on the 45th day, followed by T_4 (0.532%, 0.200% and 0.356% respectively). Overall, the 45th day emerged as the most metabolically active phase across all treatments, coinciding with peak nutrient uptake and growth (Figure 3). This supports previous studies suggesting that the mid-culture stage is ideal for nutrient assimilation due to heightened cellular division and metabolic activity (Weih *et al*., 2018).

3.3.2 Biochemical content

The treatment T_8 at 45 days of culturing recorded the maximum carbohydrate content (3.62%), followed by T_4 at 45 days (3.55%) and T_3 at 45 days (3.53%). The lowest level was recorded in T_1 at 60 days (2.16%). Similarly, the maximum protein content (3.28%) was recorded in 45-day-old cultures maintained with a combination of 2,4-D 2.0 mg/l + NAA 2.0 mg/l + BAP 2.0 mg/l. The maximum phenol content was also recorded in T_8 at 60 days (0.272%), followed by T_8 at 45 days (0.254%) and T_8 at 30 days (0.240%). The minimum values were observed in T_1 at 45 days (0.057%), 30 days (0.058%), and 60 days (0.060%) (Figure 4).

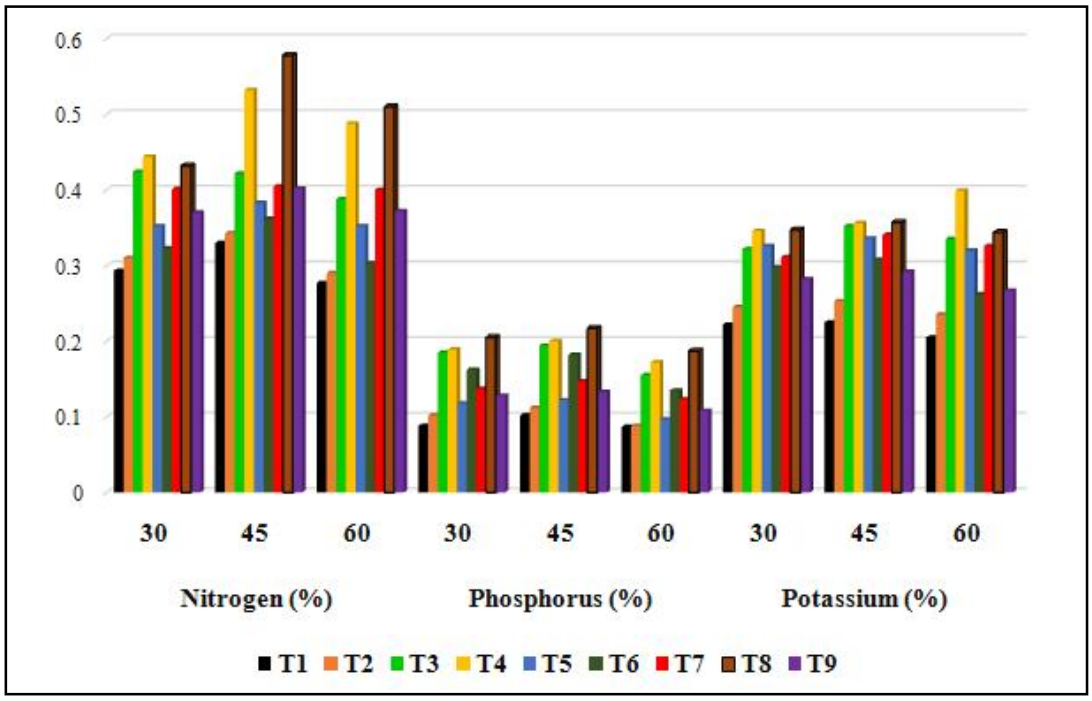

Figure 3: Influence of PGRs on NPK content (%) of suspension cells in *A. echioides*.

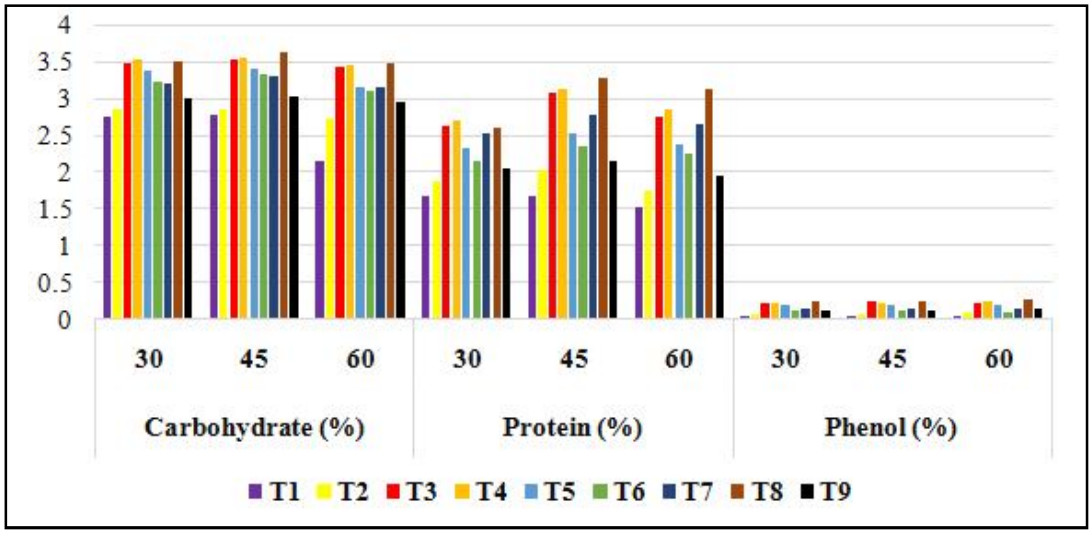

Figure 4: Influence of PGRs on biochemical components (%) of suspension cells in *A. echioides*.

3.4 Influence of sucrose concentration on nutrient content and biochemical components

3.4.1 NPK content

The highest nitrogen content (0.578%) was recorded in T_8 (2.0 mg/l 2,4-D, NAA, BAP with 3% sucrose) on the 45th day, followed by T_4 (0.560%) and T_3 (0.512%) on the same day. Phosphorus content peaked at 0.217% in T_8 (3% sucrose) on the 45th day, followed closely by T_3 (0.199%) and T_4 (0.200%). Maximum potassium accumulation (0.357%) was observed in T_8 at 45 days, with T_4 (0.356%) and T_3 (0.341%) trailing closely (Figure 5).

3.4.2 Biochemical content

The interaction effect between sucrose concentration and culture duration was significant, with the highest carbohydrate content observed in T_9 with 4% sucrose at 45 days (4.44%), and followed by T_9 with 4% sucrose at 30 days (4.32%) and 60 days (4.11%). However, the highest protein content recorded in T_8 with 3% sucrose at 45 days (3.28%), followed by T_4 with 3% sucrose at 45 days (3.12%), and T_7 with 3% sucrose at 45 days (3.01%). Whereas, the suspension cells in T_9 with 4% sucrose for 60 days recorded the highest phenol content (0.318%), followed by T_9 at 45 days (0.305%) and T_5 at 60 days (0.295%). Contrastingly, all the biochemical contents were observed lowest in T_1 across all time intervals (Figure 6).

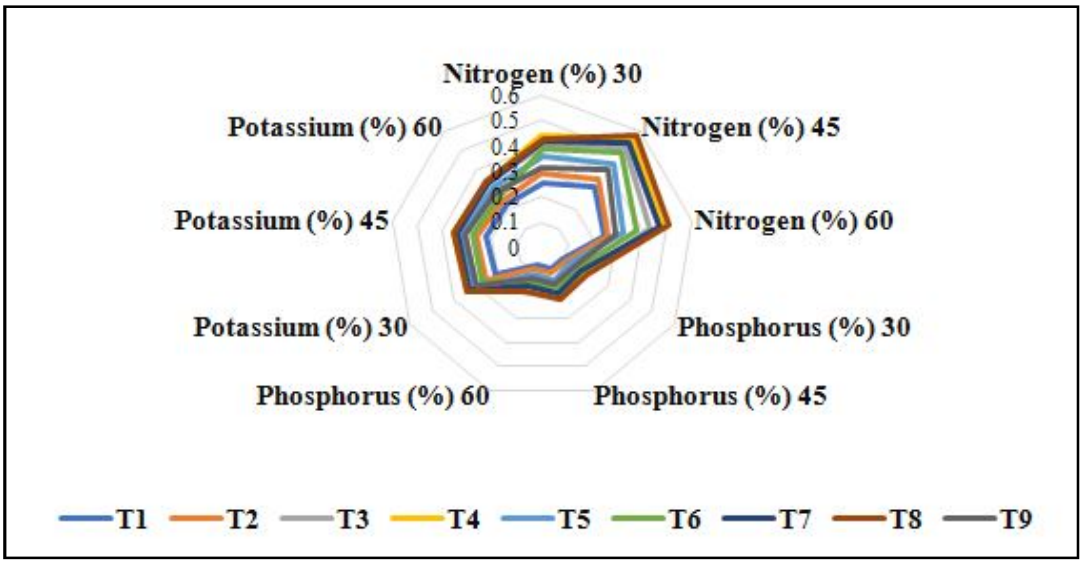

Figure 5: Influence of sucrose concentrations on NPK content (%) of suspension cells in A. echioides.

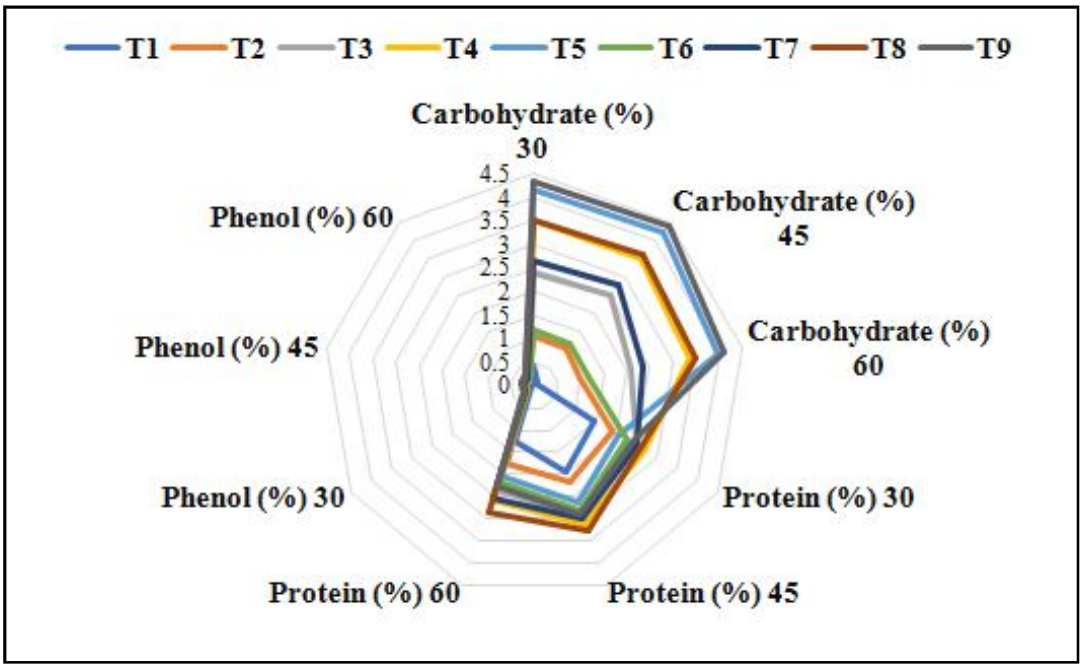

Figure 6: Influence of sucrose concentrations on biochemical components (%) of suspension cells in *A. echioides*.

Table 6: Estimation of andrographolide content (%) of suspension cells in *A. echioides*

Treatments	Andrographolide (%) days		T-mean
	45	60	
T_1	0.04	0.07	0.06
T_2	0.19	0.23	0.21
T_3	0.08	0.11	0.09
T_4	0.19	0.24	0.22
T_5	0.15	0.21	0.18
T_6	0.12	0.19	0.16
T_7	0.09	0.11	0.10
T_8	0.60	0.66	0.63
T_9	0.18	0.23	0.20
D-mean	0.18	0.23	0.21
Interaction effect	SEd	CD (0.05)	
T	0.004	0.009	
D	0.002	0.004	
TD	0.006	0.012	

* T-treatments, D-days.

3.5 Quantification of andrographolide content in suspension cells

The interaction effects of growth regulator treatments, sucrose concentrations, and culture duration, the maximum andrographolide content (0.66%) was recorded in T_8 with 3% sucrose at 60 days, which was statistically on par with 45 days (0.60%) under the same treatment (Table 6). The lowest andrographolide content was recorded in the control (MS basal) at both 45 days (0.04%) and 60 days (0.07%) of culturing.

4. Discussion

Secondary metabolites like alkaloids, terpenoids, flavonoids, phenolics, and saponins are essential for plant defense mechanisms, helping them withstand biotic and abiotic stressors including pest and pathogen attacks, environmental fluctuations, and fire damage (Upadhyay *et al.,* 2025; Al-Khayri *et al.,* 2023). Beyond their ecological functions, these compounds hold immense pharmaceutical potential due to their efficacy in treating a wide spectrum of human diseases (Deepikakrishnaven *et al.,* 2024; Vasanthkumar *et al.,* 2024). However, uncontrolled harvesting from wild and uncultivated habitats has resulted in a decline in natural populations of these plants and diminished the availability of their valuable bioactive compounds. Given these challenges, plant tissue culture-particularly cell suspension cultures-serves as a reliable and eco-friendly strategy for the uninterrupted, high-volume generation of targeted secondary metabolites in precisely regulated, and sterile environments (Wyk and Wink, 2017; Gandi *et al.,* 2012). Suspension culture offers advantages in both increasing the yield and improving the quality of metabolites, while also being cost-efficient, as cultured cells typically accumulate higher concentrations of active compounds compared to whole plants (Abdulhafiz *et al.,* 2022).

The results of this investigation clearly highlight the importance of fine-tuning plant growth regulator combinations and sucrose levels to enhance growth efficiency and nutrient assimilation in *A. echioides* suspension cultures. Among the treatments tested, T_8-comprising 2.0 mg/l each of 2,4-D, NAA, and BAP with 3% sucrose-consistently outperformed all others. This treatment resulted in the highest packed cell volume, wet and dry biomass, and peak nitrogen, phosphorus, and potassium accumulation, particularly on the 45th day of culture. This suggests a synergistic interaction between auxins and cytokinins that stimulates rapid cell proliferation, activates metabolic pathways, and enhances nutrient uptake (Reshi *et al.,* 2013; Schaller *et al.,* 2015).

Plant growth regulators like 2,4-D, NAA, BAP, and kinetin are frequently employed to initiate callus formation in a range of plant species, and MS medium supplemented with these hormones is extensively applied for *in vitro* propagation and metabolite synthesis (Turgut-Kara and Ari, 2006; Misawa, 1994). Similarly, sucrose concentration played a vital role in culture success. The 3% sucrose level emerged as optimal, providing necessary energy for biosynthetic processes and maintaining osmotic equilibrium critical for cell viability and function (Koch, 2004). In contrast, higher concentrations (e.g., 4%) likely induced osmotic stress, impairing cellular growth and nutrient transport (Mauro *et al*., 2003).

Furthermore, excessive PGR concentrations (2.5 mg/l) appeared to disturb hormonal balance, reducing overall physiological efficiency. The observed peak in biomass and nutrient accumulation at 45 days indicates this period as the most metabolically active phase of the culture. Beyond this point, signs of senescence and nutrient limitation may contribute to reduced growth and productivity, similar to trends reported in other medicinal plants (Jaishankar and Srivastava, 2017). These observations are consistent with earlier findings, such as those of Sharma and Jha (2012) who reported optimal callus induction in *A. paniculata* using 1.0 mg/2,4-D + NAA. Additionally, Pliankong *et*

al. (2018) successfully enhanced alkaloid production, including vincristine and vinblastine, in suspension cultures of *Catharanthus roseus* using MS liquid medium with 1.5 mg/l 2,4-D, 0.5 mg/l kinetin, and 3% sucrose.

The experimental data further demonstrate that nitrogen, phosphorus, and potassium accumulation depended heavily on the specific PGR regimens and sucrose concentrations applied. The maximum nitrogen content (0.578%) was observed under T_8 on the 45th day, indicating a strong correlation between the growth phase and nutrient assimilation. Phosphorus and potassium followed a similar pattern, peaking at 0.217% and 0.357% respectively, under the same treatment and time point. The mid-phase of the culture cycle (45 days) thus represents the optimal window for harvesting biomass with maximum nutrient and potential metabolite content. These results corroborate previous literature suggesting that active cell division and metabolism during this phase lead to heightened nutrient uptake and biosynthetic activity (Weih *et al.,* 2018; Palmer and Clegg, 2019; Sharma *et al.,* 2013).

Biochemical profiling of *A. echioides* suspension cultures revealed that variations in plant growth regulators and sucrose levels had a notable impact on the accumulation of carbohydrates, proteins, and phenolic compounds. The T_8 treatment (2.0 mg/l 2,4-D + 2.0 mg/l NAA + 2.0 mg/l BAP with 3% sucrose) consistently recorded the highest levels of all three biochemical components, especially at 45 days of culture. Carbohydrates and proteins peaked at 45 days, indicating active metabolic and growth phases, while phenol content gradually increased, reaching a maximum at 60 days, possibly due to stress-related secondary metabolism. These findings highlight the effectiveness of optimized hormonal and carbon source combinations in enhancing biochemical yields in *in vitro* cultures.

The quantification of andrographolide, a key therapeutic diterpene lactone, revealed significant variations in its accumulation across different combinations of PGRs, sucrose concentrations, and culture durations. Among all treatments, the combination of 2,4-D (2 mg/l), NAA (2 mg/l), BAP (2 mg/l), and 3% sucrose (T_8) consistently yielded the highest andrographolide content in suspension cultures (0.66%). These findings indicate that a balanced auxin-cytokinin ratio, combined with an optimal carbon source, plays a critical role in stimulating secondary metabolite biosynthesis in *A. echioides*. The increase in andrographolide content with culture age, particularly up to 60 days, suggests that extended culture duration facilitates the accumulation of bioactive compounds, likely due to enhanced differentiation or metabolic activity. This trend aligns with previous reports, which highlight the importance of culture maturity and hormonal signaling in activating biosynthetic pathways (Sharma and Jha, 2012; Jeandet *et al.,* 2022). Furthermore, the superior performance of 3% sucrose supports its dual role as a carbon source and osmotic agent, promoting nutrient uptake and metabolite production. The comparatively lower yields in control (MS basal) and less effective PGR combinations emphasize the need for precise hormonal and nutritional optimization in *in vitro* systems. A previous study by Vidyalakshmi and Ananthi (2013) analyzed the andrographolide content in *A. paniculata* leaves and recorded a content of 34.6 mg/g in leaves treated with IAA, compared to 5.8 mg/g in the control. Gandi *et al*. (2013) observed an andrographolide concentration of 1.53 mg/g on the 18th day of culture, suggesting that optimal andrographolide production in *A. paniculata* can be attained within a relatively short cultivation period.

5. Conclusion

The present study clearly shows that both the type and concentration of PGRs and sucrose in the culture medium have a significant impact on the growth and nutrient content of suspension cells in *A. echioides*. Among all treatments, the combination of 2.0 mg/l of each 2,4-D, NAA, and BAP with 3% sucrose was the most effective, leading to the highest biomass accumulation and optimal uptake of essential macronutrients-nitrogen, phosphorus, and potassium. The synergistic interaction between auxins and cytokinins, along with an ideal carbon source, created a favourable environment for enhanced cell proliferation and metabolic activity. Furthermore, the study identified 45 days as the most productive culture period, marking the peak phase for cellular growth and nutrient assimilation. This optimized condition also resulted in the maximum accumulation of andrographolide, a key bioactive compound, underscoring the protocol's potential for pharmaceutical applications. These findings provide a scientifically validated protocol for maximizing *in vitro* biomass production, nutrient enrichment, and secondary metabolite synthesis in *A. echioides*, which can be applied to large-scale tissue culture, pharmaceutical compound production, and advanced metabolic studies. Future research could focus on the integration of elicitors or bioreactor systems to further enhance secondary metabolite synthesis under these optimized culture conditions.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Abdulhafiz, F.; Mohammed, A.; Reduan, M.F.H.; Kari, Z.A.; Wei, L.S. and Goh, K.W. (2022). Plant cell culture technologies: A promising alternatives to produce high-value secondary metabolites. Arabian Journal of Chemistry, **15**(11):104161.

Al-Khayri, J.M.; Rashmi, R.; Toppo, V.; Chole, P.B.; Banadka, A.; Sudheer, W.N.; Nagella, P.; Shehata, W.F.; Al-Mssallem, M.Q.; Alessa, F.M. and Almaghasla, M.I. (2023). Plant secondary metabolites: The weapons for biotic stress management. Metabolites, **13**(6):716.

Andrographolide Market Report (2025). Andrographolide market report 2025 (global edition). https://www.cognitivemarketresearch.com/andrographolide-market-report, Cited on 12th May, 2025.

Basu, S.K.; Rupeshkumar, M. and Kavitha, K. (2009a). Hepatoprotective and antioxidant effect of *Andrographis echioides* N. against acetaminophen induced hepatotoxicity in rats. Journal of Biological Sciences, **9**:351-356.

Basu, S.K.; Rupeshkumar, M. and Kavitha, K. (2009b). Studies on the anti-inflammatory, analgesic and antipyretic properties of *Andrographis echioides* Nees. International Journal of Pharmacology, pp:5.

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principles of protein dye binding. Analytical Biochemstry, **72**:248-254.

Cui, X. H.; Murthy, H. N.; Wu, C.H. and Paek, K.Y. (2010). Sucrose-induced osmotic stress affects biomass, metabolite, and antioxidant levels in root suspension cultures of *Hypericum perforatum* L. Plant Cell, Tissue and Organ Culture (PCTOC), **103**(1):7-14.

Deepikakrishnaveni, T.; Jaya Jasmine, A.; Ganga, M.; Rajakumar, D. and Senthil, A. (2024). A review on the nutritional composition and pharmacological properties of ornamental plants beyond their aesthetic and therapeutic potential. Ann. Phytomed., **13**(2):55-62.

Gandi, S.; Rao, K.; Chodisetti, B. and Giri, A. (2012). Elicitation of andrographolide in the suspension cultures of *Andrographis paniculata*. Applied biochemistry and biotechnology, **168**:1729-1738.

Harborne, J.B. (1973). Phytochemical methods. Jechmonn and Hall, London, pp:90.

Hedge, J.E. and Hofreiter, B.T. (1962). Carbohydrate chemistry. (Eds.) Whistler, R.L. and Miller, B.C. J.N. Academic Press New York, **17**:371-380.

Jaishankar, J. and Srivastava, P. (2017). Molecular basis of stationary phase survival and applications. Frontiers in Microbiology, **8**:123-128.

Jeandet, P.; Formela-Luboińska, M.; Labudda, M. and Morkunas, I. (2022). The role of sugars in plant responses to stress and their regulatory function during development. International Journal of Molecular Sciences, **23**(9).

Koch, K. (2004). Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Current Opinion in Plant Biology, **7**(3):235-246.

Kumar, P.; Kumar, T.; Singh, S.; Tuteja, N.; Prasad, R. and Singh, J. (2020). Potassium: A key modulator for cell homeostasis. Journal of Biotechnology, **324**:198-210.

Kumaran, K.S.; Thirugnanasambantham, P.; Viswanathan, S. and Murthy, M.S.R. (2003). An HPLC method for the estimation of andrographolide in rabbit serum. Indian Journal of Pharmacology, **35**:109-112.

Li, X.; Yuan, W.; Wu, J.; Zhen, J.; Sun, Q. and Yu, M. (2022). Andrographolide, a natural anti-inflammatory agent: An Update. Frontiers in Pharmacology, **13**:920435.

Maheshwari, M.; Ramkumar R.; Arun V.P.; Anandharaj, B. and Sathya, M. (2021). Evaluation of phytochemical analysis and antibacterial activity of *Andrographis echioides* (L) nees (Green chiretta). International Journal of Botany Studies, **6**(4):378-383.

Mauro, M.A.; de Queiroz Tavares, D.; and Menegalli, F.C. (2003). Behavior of plant tissue in osmotic solutions. Journal of Food Engineering, **56**(1):1-15.

Ohyama, T. (2010). Nitrogen as a major essential element of plants. In Nitrogen Assimilation in Plants, pp:1-18.

Palmer, B.F. and Clegg, D.J. (2019). Physiology and pathophysiology of potassium homeostasis: core curriculum 2019. American Journal of Kidney Diseases, **74**(5):682-695.

Pliankong, P.; Suksa-Ard, P. and Wannakrairoj, S. (2018). Chitosan elicitation for enhancing of vincristine and vinblastine accumulation in cell culture of *Catharanthus roseus* (L.) G. Don. Journal of Agricultural Science, **10**(12):287-293.

Raja, R.R. and Jeevanreddy, K. (2014). Pharmacognostical phytochemical and antiulcer activity of *Andrographis echioides* (Acanthaceae). Journal of Pharmacognosy and phytochemistry, **3**(3):39-49.

Reshi, N.A.;Sudarshana, M.S. and Rajashekar, N. (2013). Callus induction and plantlet regeneration in *Orthosiphon aristatus* (Blume) Miq-apotent medicinal herb. IOSR Journal of Pharmacy and Biological Sciences, **6**(3):52-55.

Schaller, G. E.; Bishopp, A. and Kieber, J. J. (2015). The yin-yang of hormones: Cytokinin and auxin interactions in plant development. Plant Cell, **27**(1):44-63.

Sharma, N.; Vandana, M.; Kumar, C. and Choudhary, R. C. (2013). Effect of 2,4-D, BAP, KN, IAA, and IBA on *in vitro* regeneration of *Ocimum canum* Sims : An important hoary basil plant. International Journal of Agriculture, Environment and Biotechnology, **6**:389-395.

Sharma, S.N. and Jha, Z. (2012). Production of andrographolide from callus and cell suspension culture of *Andrographis paniculata*. Journal of Cell and Tissue Research, **12**(3).

Tian, T.; Chu, X.Y.; Yang, Y.; Zhang, X.; Liu, Y. M.; Gao, J.; Ma, B. G. and Zhang, H.Y. (2019). Phosphates as energy sources to expand metabolic networks. Life, **9**(2).

Turgut-Kara, N. and Arý, S. (2006). Micropropagation of *Astragalus maximus* willd. Biotechnology and Biotechnological Equipment, **20**(1):20-22.

Upadhyay, R.; Saini, R.; Shukla, P.K. and Tiwari, K.N. (2025). Role of secondary metabolites in plant defense mechanisms: A molecular and biotechnological insights. Phytochemistry Reviews, **24**(1):953-983.

Vasanthkumar, S. S.; Prabhu, T.; Shenbagavalli, S.; Rajangan, J.; VallalKannan, S. and Baskaran, A. (2024). Exploring the therapeutic potential of bioactive compounds in spices and herbs: Preclinical evidence and medicinal applications. Ann. Phytomed., **13**(2):230-237.

Vidyalakshmi, A. and Ananthi, S. (2013). Induction of andrographolide, a biologically active ingredient in callus of *Andrographis paniculata* (Burm. F) Wallich Ex. Nees. Bioeng Biosci, **1**(1):1-4.

Weih, M.; Hamnér, K. and Pourazari, F. (2018). Analyzing plant nutrient uptake and utilization efficiencies: Comparison between crops and approaches. Plant and Soil, **430**(1):7-21.

Wyk, V. B. and Wink, M. (2004). Medicinal plants of the world: An illustrated scientific guide to important medicinal plants and their uses. Timber Press, Portland, pp:480.

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