Analysis of the effect of organic supplements on the growth of chrysanthemum explants (*Chrysanthemum* sp.) in tissue culture media

Abdul Haris[1]*, Abdullah[1], Aminah[1], Abdul Akbar[1], Dita Dindasari[1]

[1]Agrotechnology Study Program, Faculty of Agriculture, Universitas Muslim Indonesia, Jl. Urip Sumoharjo No.km.5, Panaikang, Panakkukang District, Makassar City, South Sulawesi 90231.
*Corresponding author: abdul.haris@umi.ac.id

Received: March 21st, 2024 / Accepted: September 19th, 2024

ABSTRACT

Chrysanthemum (*Chrysanthemum* sp.) is an ornamental plant of high economic value with a continuously increasing demand, necessitating efficient production methods. Tissue culture techniques are one of the solutions that enable rapid and disease-free plant propagation. This study aims to evaluate the effect of organic supplements on the growth of chrysanthemum explants. The research was conducted using a completely randomized design with six treatments, namely control (without supplements), banana extract, corn seed extract, coconut water, and combinations of coconut water with banana and corn seed extracts. The results show that the banana extract supplement at a concentration of 50 g/L provides the best growth in terms of plantlet height, number of leaves, and roots compared to other treatments. The combination of coconut water and banana extract also significantly enhances growth. This study concludes that the use of organic supplements can improve the efficiency of chrysanthemum propagation through tissue culture, supporting more sustainable ornamental plant production.

Keywords: banana extract, coconut water, explant growth, horticulture, organic supplements, tissue culture.

INTRODUCTION

Organic supplements in tissue culture media for the growth of chrysanthemum explants (Chrysanthemum sp.) are crucial considering the popularity and commercial value of this plant. Chrysanthemums are known as ornamental plants with beautiful flowers of various colors, leading to a continuous increase in demand in both local and international markets. To meet this demand, the horticulture sector needs to enhance the production and quality of chrysanthemum plants. One effective method to achieve this goal is through tissue culture techniques, which allow for rapid and large-scale plant propagation, resulting in disease-free plants (Apriliani, 2023).

Previous research has shown that the addition of organic supplements to culture media can improve explant growth. For example, the use of coconut water as an organic supplement in banana culture showed better results compared to the control, with improvements in growth parameters such as plant height and the number of shoots (Tuwo et al., 2021). This indicates the potential of organic supplements to enhance the efficiency of tissue culture, which can be applied to chrysanthemum plants to improve yield and quality. Furthermore, research conducted by Murgayanti et al. demonstrated that the use of cytokinins in tissue culture can stimulate shoot and root growth, which is also relevant for the development of tissue culture techniques for chrysanthemums (Murgayanti et al., 2020).

The use of appropriate culture media is also crucial for the success of plant propagation. Modifying media with the addition of plant growth regulators (PGRs) can increase germination rates and plant growth (Pratiwi et al., 2020). In the context of chrysanthemums, selecting nutrient-rich media and adding suitable organic supplements can support explant growth, resulting in high-quality seedlings. The appropriate concentration of hormones can significantly affect explant growth, indicating that regulating culture conditions is vital in plant propagation (Prasayu & Ratnasari, 2021).

In efforts to improve the quality and quantity of chrysanthemum production, this study aims to explore the effects of various organic supplements in tissue culture media on the growth of chrysanthemum explants. By understanding how organic supplements can influence explant growth, it is hoped that optimal combinations can be found to enhance tissue culture efficiency. This research is expected to make a significant contribution to the development of tissue culture techniques for chrysanthemum plants, as well as support sustainability and increased production in the horticulture sector (Nofrianinda et al., 2018).

MATERIALS AND METHODS

This research was conducted from March to April 2018 at the Plant Tissue Culture Laboratory, Technical Implementation Unit of the Horticultural Seed Center of South Sulawesi Province, Bonto-Bonto, Gowa District.

The materials used in this study included 8-10 week-old cuttings of Bacardi chrysanthemum plants, sterile aquadest, Murashige & Skoog (MS) medium components: NH4NO3, KNO3, CaCl2 x 2H2O, MgSO4 7 H2O, KH2PO4, KI,

DOI: https://doi.org/10.21107/agrovigor.v17i2.27630

Agrovigor: Jurnal Agroekoteknologi
http://journal.trunojoyo.ac.id/agrovigor
ISSN: 1979-5777 (*Print*), 2477-0353 (*Online*)

H3BO3, MnSO4 7 H2O, ZnSO4 7H2O, Na2MoO4 2H2O, CuSO4 5H2O, CoCl2 6H2O, Nicotinic acid, pyridoxine HCl, Thiamine HCl, Glycine, FeSO4 7H2O, Na2 EDTA, Myo Inositol (see Appendix Table 1), perfectly ripe 'raja' bananas, young coconut water, young sweet corn, granulated sugar, gelling agent (agar Swallow), clear plastic, rubber bands, tissue, plastic wrap, 70% and 90% alcohol.

The equipment used included an analytical balance, Erlenmeyer flasks, graduated cylinders, beakers, spatulas, pH meter, petri dishes, pots, gas stove, blender, sprayer, culture/media bottles, autoclave, culture knives, tweezers, Bunsen burner, laminar air flow cabinet, and scissors.

This research was conducted using a completely randomized design (CRD) consisting of 6 treatments, as follows:

M0 = MS + without organic supplements (control)
M1 = MS + banana extract supplement at a concentration of 50 g/L
M2 = MS + sweet corn seed extract supplement at a concentration of 100 g/L
M3 = MS + coconut water supplement at a concentration of 100 mL/L
M4 = MS + 100 mL coconut water + 50 g/L banana extract
M5 = MS + 100 mL coconut water + 100 g/L corn seed extract

Each treatment was replicated 10 times, resulting in 60 experimental units, and each treatment unit was arranged randomly. The research data were analyzed based on the F-test (0.5% and 1% significance levels), and if there were significant effects, further testing was conducted using the Honest Significant Difference (HSD) test at the 0.05 level.

Implementation of the Research

1. **Sterilization of Tools and Work Environment**
   The tools used must be free from contaminants or in a sterile condition. Metal and glass tools, as well as aquadest, are sterilized in an autoclave at a temperature of 121°C and a pressure of 17.5 psi for 60 minutes. Glassware (petri dishes, graduated cylinders) is wrapped in paper before autoclaving. Tools such as tweezers and scissors are sterilized by burning in 96% alcohol. The working surface of the Laminar Air Flow Cabinet is sterilized by spraying 75% alcohol and then cleaned with tissue or by turning on the ultraviolet lamp for 30 to 60 minutes.
2. **Preparation of Stock Solutions**
   To facilitate the preparation of tissue culture media, the components of the media are made in the form of stock solutions. The stock solution for MS medium is prepared according to the composition and concentration listed in Appendix Table 1. The stock solutions are prepared based on the types of medium components and are labeled from A to H (NH4NO3, KNO3, CaCl2 x 2H2O, MgSO4 7 H2O, KH2PO4, KI, H3BO3, MnSO4 7 H2O, ZnSO4 7H2O, Na2MoO4 2H2O, CuSO4 5H2O, CoCl2 6H2O, Nicotinic acid, pyridoxine HCl, Thiamine HCl, Glycine, FeSO4 7H2O, Na2 EDTA, Myo Inositol). The chemicals are weighed and each is dissolved in one liter of sterile aquadest. The solution is then placed in an Erlenmeyer flask, covered with aluminum foil, and labeled. Specifically for stock solution F (FeSO4 7H2O, Na2 EDTA), the Erlenmeyer flask is entirely wrapped in aluminum foil to avoid light exposure. The stock solutions are then stored in the refrigerator.
3. **Preparation of Organic Extracts**
   Organic materials such as 'raja' bananas, sweet corn, and young coconut water used in the media preparation must be in good and ripe condition. Before use, the bananas and sweet corn are sprayed with 70% alcohol, while the coconut water is filtered using filter paper. The sweet corn kernels are weighed at 100 g, then blended until smooth, and the corn extract is filtered before adding 100 mL of aquadest and then incorporated into the MS medium. The banana extract is prepared by slicing the bananas, weighing 50 g each, blending them with 100 mL of aquadest, and then filtering the mixture. The prepared extract is mixed into 1 liter of MS medium.
4. **Preparation of Treatment Media**
   The MS medium is made by pipetting the appropriate amounts of the prepared stock solutions into a 1000 mL volumetric flask. After all medium components are added, the corn extract (100 g/L), banana extract (50 g/L), and coconut water (100 mL/L) are included. Then, 30 g of granulated sugar and one packet of agar are added, and the solution is brought to a final volume of 1000 mL with aquadest. The pH of the culture medium is measured by dipping a pH meter into the solution and should be set around 5.8 to 6. If the pH is low, NaOH (sodium hydroxide) is added, and if the pH is high, HCl (hydrochloric acid) is added to lower the pH to the desired level. The mixed medium solution is then placed in a flask and boiled. After boiling, it is removed and poured into culture bottles with a volume of 25 mL each, sealed with clear plastic and secured with rubber bands, and then sterilized in an autoclave for 15 minutes at 121°C with a pressure of 17 psi.
5. **Planting**
   Explant planting is carried out in the Laminar Air Flow Cabinet using plant materials (cuttings) from the tissue culture laboratory, which are cut into sections with one node and placed in sterile petri dishes. The explants are then planted in the prepared media according to the treatments being tested. Each bottle consists of 3 cuttings. After planting, the bottles are sealed and stored on the culture rack with light intensity between 1000 lux and 4000 lux.
6. **Maintenance**
   The lighting provided to the plants depends on the plant type and the desired response. Continuous lighting is applied to chrysanthemum plants, with an average temperature maintained between 18°C and 20°C. Additionally, cleanliness in the culture room must be maintained to avoid contamination.
7. **Observation**
   Observations are conducted for 3 weeks to evaluate the response of chrysanthemum plants to the organic supplements added to the culture media, with the following observations conducted:

1. Root Initiation Time (Days After Planting - DAP)
   Observing the time it takes for roots to grow and form on chrysanthemum explants by examining the bottom of the culture bottles from the outside. Observations are conducted daily after planting until roots appear.
2. Plantlet Height (cm)
   The height of the plant is measured from the surface of the medium to the tip of the highest leaf, by measuring the plant's height from outside the culture bottle. Measurements are taken at the end of the study (21 days after planting).
3. Number of Leaves (leaves)
   Counting the number of fully developed leaves at the end of the study (21 days after planting).
4. Number of Roots
   The number of roots is counted at the end of the study (21 days after planting).
5. Number of Shoots
   Counting the number of shoots that have formed at the end of the study (21 days after planting).

RESULTS AND DISCUSSION

Root Initiation Time (Days After Planting)

The average observation results of root initiation time and its variance analysis are presented in Table Appendix 2a and 2b. The variance analysis indicates that the type of plant extract in the treatment medium has a highly significant effect on the root initiation time.

The results of the HSD test at the 0.05 level in Table 1 show that the treatment with king banana extract supplement at a concentration of 50 g/l (M1) and without supplement (M0) had a faster effect on root initiation time. In contrast, the treatment with sweet corn kernel extract at a concentration of 100 g/l (M2) and the combination of coconut water 100 ml + sweet corn kernel extract 100 g/l (M5) had a slower effect on the average root initiation time. The results of the Honest Significant Difference (HSD) test at the 0.05 confidence level indicate that the treatment with king banana extract supplement at a concentration of 50 g/l (M1) and without supplement (M0) had a faster effect on root initiation time. This is consistent with research showing that banana extract can enhance root and shoot growth in various plant types, including orchids and other horticultural plants (Permatasari et al., 2022). Banana extract has the potential to improve vegetative growth, which may contribute to a quicker root initiation time (Yulianti et al., 2016).

The treatment with sweet corn kernel extract at a concentration of 100 g/l (M2) and the combination of coconut water 100 ml + sweet corn kernel extract 100 g/l (M5) had a slower effect on the average root initiation time. The use of corn extract at high concentrations can inhibit root growth, which may explain the results obtained in this treatment (Sakinah et al., 2023). Additionally, while coconut water has benefits for growth, it may interact with other treatments, possibly resulting in slower growth under certain conditions (Sutoto et al., 2021).

Table 1. Average Root Initiation Time (Days) for Various Plant Extracts as Supplements in Tissue Culture Medium

Treatment	Average value	HSD 0,05
	Waktu Inisisasi Akar	
M0 (without supplement)	5,00 a	
M1 (king banana extract 50 g/l)	5,00 a	
M2 (sweet corn kernel extract 100 g/l)	10,60 b	2,36
M3 (coconut water 100 ml/l)	5,60 a	
M4 (coconut water 100 ml + king banana extract 50 g/l)	6,20 a	
M5 (coconut water 100 ml + sweet corn kernel extract 100 g/l)	11,00 b	

Note: Average values followed by different letters (a, b) are significantly different at the HSD test level (0.05).

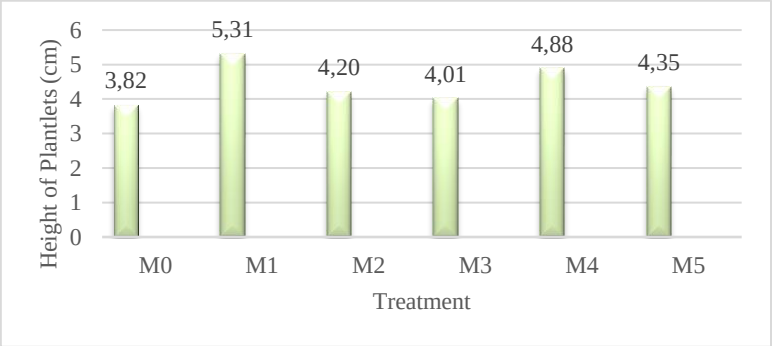

Figure 1. Average height of plantlets at 21 days after planting using various plant extracts as supplements in tissue culture media.

Height of Plantlets (cm)

The results of the average plantlet height observations at 21 days after planting (hst) and their variance analysis are presented in the table below. The data indicate that the type of extract in the treatment media had no significant effect on plantlet height.

The graph shows the variation in plantlet height (cm) based on six different treatments, namely M0 to M5. The lowest plantlet height was recorded for treatment M0 at 3.82 cm, while treatment M1 resulted in the highest plantlet height of 5.31 cm. Treatments M2 and M3 produced plantlet heights of 4.20 cm and 4.01 cm, respectively. Meanwhile, treatment M4 showed a plantlet height close to M1 at 4.88 cm, and M5 resulted in a height of 4.35 cm. Thus, it can be seen that treatment M1 provides the most optimal result for plantlet height compared to other treatments.

The observation of plantlet height based on the six different treatments, namely M0 to M5, showed significant variation. Treatment M0, which received no supplements, recorded the lowest plantlet height of 3.82 cm. In contrast, treatment M1, which used banana extract at a concentration of 50 g/l, resulted in the highest plantlet height of 5.31 cm. Previous research has shown that banana extract can enhance plant growth, including plantlet height, due to the nutrient content and growth hormones that support physiological processes in plants (Arum & Semiarti, 2022; Rahayu et al., 2021).

Treatments M2 and M3 produced plantlet heights of 4.20 cm and 4.01 cm, respectively, indicating that although there was an increase compared to M0, both were not as effective as M1. Treatment M4, which is close to M1 with a height of 4.88 cm, suggests that a combination of treatments can yield better results, although it does not reach the optimal level achieved by M1. Treatment M5 resulted in a height of 4.35 cm, showing that although there was an increase, the results were still lower compared to treatment M1 (Abdurhman et al., 2022).

From these results, it can be concluded that treatment M1 provides the most optimal result for plantlet height compared to other treatments. This aligns with studies showing that the use of banana extract in tissue culture can significantly enhance the growth and development of plantlets (Islam et al., 2016). Therefore, the selection of the type and concentration of supplements is crucial in enhancing plantlet growth in tissue culture.

Number of Leaves (pieces)

The table shows the effect of various treatments on the number of leaves in planlets. Treatment M0 (without supplement) resulted in the lowest average number of leaves at 5.20, which is statistically significantly different, indicated by the letter "b." Treatment M1 (banana peel extract at a concentration of 50 g/l) produced the highest number of leaves with an average of 6.80, followed by M4 (coconut water at 100 ml + banana peel extract at 50 g/l), which yielded an average of 7.70. Treatments M2 (sweet corn seed extract at 100 g/l), M3 (coconut water at 100 ml/l), and M5 (coconut water at 100 ml + sweet corn extract at 100 g/l) produced average leaf counts of 5.60, 6.40, and 6.50, respectively, which did not show statistically significant differences compared to M1 and M4. These results indicate that the use of supplements, particularly the combination of coconut water and banana extract, significantly increases the number of leaves compared to the treatment without supplements (M0) at the BNJ test level of 0.05.

Observations of the number of leaves in planlets based on various treatments show that treatment M0 (without supplement) resulted in the lowest average leaf count of 5.20, which is statistically significantly different, indicated by the letter "b." In contrast, treatment M1, which used banana peel extract at a concentration of 50 g/l, produced the highest number of leaves with an average of 6.80. Treatment M4, which is a combination of 100 ml of coconut water and 50 g/l of banana peel extract, showed even better results with an average of 7.70 leaves per planlet. Previous studies have shown that the use of banana peel extract can significantly increase leaf number and vegetative growth in plants (Fahlevi et al., 2022).

Treatments M2 (sweet corn seed extract at 100 g/l), M3 (coconut water at 100 ml/l), and M5 (coconut water at 100 ml + sweet corn extract at 100 g/l) produced average leaf counts of 5.60, 6.40, and 6.50, respectively. Although these results show an increase compared to M0, they are not statistically significantly different from M1 and M4. This indicates that while these treatments provide better results than the control, the combination of coconut water and banana peel extract has a more significant effect on increasing the number of leaves (Setiawan et al., 2021).

Table 2. Average Number of Leaves (pieces) from Various Plant Extracts as Supplements in Tissue Culture Media

Treatment	Average Value	HSD
	Number of Leaves	0,05
M0 (without supplement)	5,20 b	
M1 (banana extract 50 g/l)	6,80 a	
M2 (sweet corn extract 100 g/l)	5,60 a	2,37
M3 (coconut water 100 ml/l)	6,40 a	
M4 (coconut water 100 ml + banana extract 50 g/l)	7,70 a	
M5 (coconut water 100 ml + sweet corn extract 100 g/l)	6,50 a	

Note: Average values followed by different letters (a, b, c) indicate significant differences at the BNJ test level (0.05).

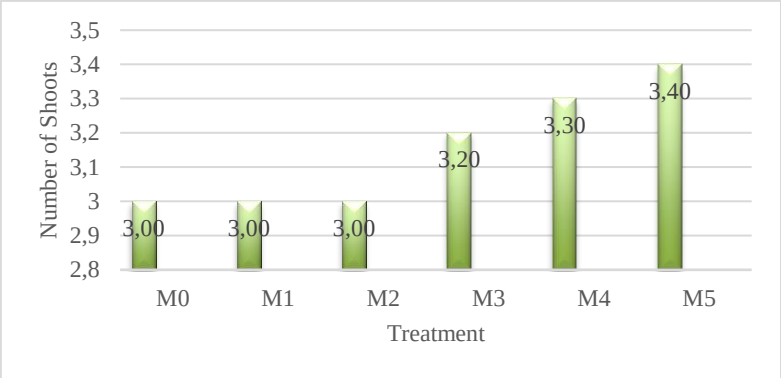

Figure 2. Average Number of Shoots from Various Plant Extracts as Supplements in Tissue Culture Media, Age 21 Days After Planting (dap)

Table 3. Average Number of Roots from Various Plant Extracts as Supplements in Tissue Culture Media

Treatment	Average Value	HSD
	Jumlah akar	0,05
M0 without supplement	6,43 d	
M1 banana extract (pisang raja) 50 g/l	9,90 b	
M2 sweet corn seed extract 100 g/l	9,27 b	1,32
M3 coconut water 100 ml/l	8,17 c	
M4 coconut water 100 ml + banana extract (pisang raja) 50 g/l	11,23 a	
M5 coconut water 100 ml + sweet corn seed extract 100 g/l	10,97 a	

Note: The average values followed by different letters (a, b, c, d) indicate a significant difference at the BNJ test level (0.05).

These results are consistent with studies showing that the use of organic supplements, such as plant extracts and liquid fertilizers, can enhance vegetative growth in plants, including the number of leaves, which is crucial for photosynthesis and overall plant yield (Mubarok et al., 2013). Therefore, it can be concluded that the use of supplements, especially the combination of coconut water and banana extract, significantly improves the number of leaves compared to the treatment without supplements (M0) at the BNJ test level of 0.05.

2. Number of Shoots

The results of the average number of shoots and their variance analysis are presented in Tables Appendix 6a and 6b. The variance analysis indicates that the various types of plant extract supplements in the treatment media have no significant effect on the number of shoots.Figure 2 shows the average number of shoots from various treatments with plant extracts as supplements in tissue culture media at 21 days after planting (dap). Treatment M0 (without supplements), M1 (banana extract 50 g/l), and M2 (sweet corn seed extract 100 g/l) each produced the same average number of shoots, which is 3.00 shoots. Treatment M3 (coconut water 100 ml/l) showed a slight increase with an average of 3.20 shoots. Meanwhile, treatment M4 (coconut water 100 ml + banana extract 50 g/l) produced 3.30 shoots, and M5 (coconut water 100 ml + corn seed extract 100 g/l) showed the highest number of shoots at 3.40 shoots. From this data, it can be concluded that the use of a combination of coconut water and plant extracts, especially in treatment M5, provides the most optimal results in increasing the number of shoots in plant tissue culture compared to the other treatments.

These results indicate that the use of a combination of coconut water and plant extracts, particularly in treatment M5, yields the most optimal outcome in enhancing the number of shoots in plant tissue culture compared to other treatments. Previous studies have shown that coconut water contains growth hormones that can stimulate shoot formation and vegetative growth in plants (Maher et al., 2019; Tofiana et al., 2016). Additionally, banana extract is also known to increase the number of shoots and plant growth in tissue culture, thanks to its nutrient content and hormones that support physiological processes in plants (Zhang et al., 2014; Karim, 2022).

The combination of coconut water and plant extracts, as seen in treatments M4 and M5, demonstrates a positive synergy in enhancing the number of shoots. This aligns with research indicating that using nutrient-rich media and growth hormones can improve shoot proliferation in tissue culture (Kumari et al., 2015; Sharma et al., 2017). Therefore, it can be concluded that treatments with a combination of coconut water and plant extracts are very effective in increasing the number of shoots in tissue culture, which is important for mass plant propagation.

3. Number of Roots

The average number of roots observed and its variance are presented in Tables Appendix 5a and 5b. The variance shows that the application of various organic supplements has a significant effect on the number of roots.

The use of a combination of coconut water at 100 ml/l and banana extract at 50 g/l (M4) produced the highest results with an average of 11.23 roots, followed by the combination of coconut water at 100 ml/l and sweet corn seed extract at 100 g/l (M5) with 10.97 roots. Both treatments are marked

with the letter "a," indicating that they did not show significant differences. The confidence level used in the Honest Significant Difference (HSD) test is 0.05, with an HSD value of 1.32, indicating significant differences among several treatments. The use of a combination of coconut water and banana extract in this study showed significant results in enhancing root growth.

The combination of coconut water at 100 ml/l and banana extract at 50 g/l (M4) produced an average of 11.23 roots, while the combination of coconut water at 100 ml/l and sweet corn seed extract at 100 g/l (M5) yielded an average of 10.97 roots. These two treatments did not show significant statistical differences, as indicated by the letter "a" in the analysis results. This aligns with previous research indicating that the use of liquid organic materials, such as coconut water and banana extract, can enhance plant growth by providing necessary nutrients for root development (Ariyanti, 2023; Zanatia et al., 2021).

The confidence level used in the Honest Significant Difference (HSD) test is 0.05, with an HSD value of 1.32. This indicates that there are significant differences among several treatments, supporting the importance of selecting the right nutrient combinations in crop cultivation. Previous studies have also shown that the use of liquid organic fertilizers, including coconut water, can significantly improve plant growth and yield (Puspadewi et al., 2016; Saleh et al., 2023). Additionally, banana extract has been shown to have positive effects on plant growth, both as a nutrient source and as a natural growth regulator (Rahayu et al., 2021; Yulianti et al., 2016).

Overall, the results of this study confirm that the combination of coconut water and banana extract is an effective alternative for enhancing root growth in plants, which can be applied in sustainable agricultural practices. Further research is needed to explore the mechanisms behind the synergistic effects of this combination and to optimize the concentrations used in field applications (Hamidah, 2022; Ambarwati et al., 2021).

CONCLUSION

This study demonstrates that organic supplements, particularly banana extract at 50 g/l and the combination of coconut water at 100 ml/l with banana extract, significantly enhance the growth of chrysanthemum explants (Chrysanthemum sp.) compared to the control. Banana extract has proven to be the most effective in increasing plantlet height, leaf count, and root development. These results support the use of organic supplements to improve the efficiency of chrysanthemum propagation through tissue culture and contribute to more sustainable horticultural production.

CONFLICT OF INTEREST

There is no conflict of interest.

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