Growth Response and Microbulb Formation of True Shallot Seed (*Allium cepa* L.) with Sucrose Supplementation in Compound Fertilizer Medium under In Vitro Conditions

ABSTRACT

The limited availability of high-quality shallot planting material has encouraged the development of alternative propagation techniques that are disease-free through in vitro culture. The use of alternative culture media is required to improve efficiency and reduce production costs. This study aimed to evaluate the effects of various concentrations of compound NPK fertilizer and sucrose on the growth and microbulb formation of shallots derived from True Shallot Seed (TSS). The research was conducted at the Plant Tissue Culture Laboratory, Department of Agronomy, Faculty of Agriculture, University of Bengkulu, from August to November 2025. The experiment employed a factorial Completely Randomized Design (CRD) with two factors: the concentration of compound NPK fertilizer (2 g/L, 4 g/L, and Murashige and Skoog (MS) medium as the control) and sucrose concentration (30, 60, 90, and 120 g/L). Each treatment was replicated three times, with each replication consisting of three culture bottles, and each bottle planted with three TSS seeds. The main materials used were TSS seeds of the Lokananta variety and compound NPK fertilizer (32-10-10). Data were analyzed using analysis of variance (ANOVA) at the 5% significance level, followed by Duncan's Multiple Range Test (DMRT). Data with a coefficient of variation (CV) greater than 30% were transformed using the $\sqrt{(x + 0.5)}$ formula to reduce variability. The results showed a significant interaction between NPK fertilizer concentration and sucrose concentration. The combination of MS medium with 60 g/L sucrose produced the highest number of leaves and roots. MS medium resulted in the highest mean values for germination time, germination percentage, plant height, number of shoots, root length, and bulb diameter. However, the NPK compound fertilizer medium at a concentration of 2 g/L was only able to match the MS medium in terms of germination percentage and germination time.

Keywords: tissue culture, alternative medium, carbon source, in vitro shallot

1. INTRODUCTION

Shallot is one of the leading horticultural commodities in Indonesia and is widely used as a cooking ingredient as well as in traditional medicine with various health benefits (Aryanta, 2019). Shallots contain carbohydrates, sugars, proteins, fats, and various vitamins and minerals that are beneficial to human health (Waluyo and Sinaga, 2015). Shallot cultivation areas are widely distributed across many regions in Indonesia, making this commodity play an important role in the national economy (Nabila et al., 2023). Shallots have a relatively high economic value to meet domestic demand as well as international market needs. Population growth, the development of the culinary industry, and the expansion of export markets have driven an increase in shallot demand (Yassar et al., 2023).

National shallot production in 2024 reached 2,085,978 tons, increasing from 1,985,233 tons in the previous year (BPS, 2024). Shallot consumption in Indonesia also showed an upward trend during the 2018–2022 period. Average consumption increased from 2.758 kg per capita per year in 2018 to 3.024 kg per capita per year in 2022. This increase in

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consumption necessitates a corresponding increase in shallot production to meet national demand (Rinawati, 2023).

The availability of high-quality shallot planting material remains limited in cultivation practices. Farmers generally use bulbs from previous harvests as planting material. Repeated use of bulbs without proper selection increases the risk of transmitting viral, bacterial, and fungal pathogens, which ultimately reduces shallot productivity (Pangestuti and Sulistyaningsih, 2011). Therefore, efforts to produce high-quality planting material are required to improve shallot productivity and production.

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The use of True Shallot Seed (TSS) has the potential to be a solution for providing shallot planting material. TSS is relatively cheaper and required in smaller quantities. The use of TSS can reduce seed costs by up to 66.7% and increase yield to 30–40 tons/ha (Atman, 2021). However, planting material derived from TSS has relatively low germination capacity and seedling tolerance to environmental conditions (Prakoso and Alpandari, 2021). The germination percentage of TSS planted directly in the field has been reported to be less than 50% (Sopha and Basuki, 2017). The germination rate of TSS seedlings from nurseries is also relatively low, ranging from 51–56% (Sopha et al., 2017). TSS seedlings are susceptible to high temperatures and heavy rainfall, resulting in low establishment success in the field. Therefore, technologies that can improve germination and seedling resilience of TSS are required.

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An alternative approach to address these issues is the application of plant tissue culture techniques. Tissue culture is a method of plant propagation that enables the production of large numbers of plants in a relatively short time and under disease-free conditions (Basri, 2016). In tissue culture, the composition of the culture medium is a determining factor for success. Compound fertilizers can be used as alternative culture media because they are inexpensive and readily available, thereby reducing production costs. Fertilizers commonly used to promote optimal growth contain both macroelements (N, P, K, Mg, Ca, and S) and microelements (B, Cu, Fe, Mn, Zn, and Mo) (Laia, 2019). The study by Rosmaina et al. (2021) reported that the use of compound foliar fertilizer at a concentration of 2 ml/L could serve as an alternative medium to MS medium for in vitro propagation of Barangan banana, producing an average of 9.3 shoots per explant and 1.90 leaves per explant. Similarly, Trinawaty and Fitriani (2016) reported that the use of compound NPK fertilizer at a concentration of 2 g/L resulted in the best axillary shoot growth of sweet potato, indicating its potential as an alternative medium for in vitro culture.

Bawang mer The addition of sucrose to the culture medium is required as an energy source during plant growth. Under in vitro conditions, sucrose functions as a carbon source to substitute for photosynthetic products of the explant (Samudera et al., 2019). Sucrose plays a role in providing energy for respiration, regulating osmotic pressure, stabilizing membranes, and supporting new cell formation (Heriansyah, 2019). Carbohydrate concentration is an important factor in microbulb formation. Sucrose serves as the primary carbon source in tissue culture because it is naturally synthesized and translocated by plants (Trigiano and Gray, 2004). The use of high sucrose concentrations has been shown to induce microbulb formation. This is consistent with the study by Putri (2024), which reported that sucrose concentrations of 50–70 g/L enhanced growth and microbulb formation of TSS. Mardiana and Sumarji (2022) also reported that sucrose concentrations of 80–120 g/L improved plantlet growth. Furthermore, Ni'mah et al. (2012) reported that the optimum treatment for inducing potato microtubers was MS medium supplemented with 80 g/L sucrose combined with 7 mg/L kinetin.

2. MATERIALS AND METHODS

The research was conducted at the Agronomy Tissue Culture Laboratory, Department of Crop Science, Faculty of Agriculture, University of Bengkulu, from August to November 2025. The materials used in this study included True Shallot Seeds (TSS) of the Lokananta variety, compound fertilizer NPK 32-10-10, Murashige and Skoog (MS) medium, sterilizing agents (96% and 70% alcohol), sugar, 1 N HCl, 1 N NaOH, 1 N KOH, BAP, fungicide, bactericide, agar, sterile distilled water, labels, tissue paper, millimeter block paper, rubber bands, transparent plastic, and wrapping film.

The experimental design used was a factorial Completely Randomized Design (CRD) with two treatment factors. The first factor was the concentration of compound NPK fertilizer, consisting of three levels: 2 g/L, 4 g/L, and Murashige and Skoog medium as the control. The second factor was sucrose concentration, consisting of four levels: 30 g/L, 60 g/L, 90 g/L, and 120 g/L. Thus, there were 12 treatment combinations, each replicated three times, resulting in 36 experimental units. Each replication consisted of three culture bottles, and each bottle contained three TSS seeds.

The experimental stages included sterilization of equipment and the working environment using NaOCl solution, alcohol, and formalin, followed by autoclaving at 121°C and 15 psi pressure for 30 minutes. The culture media were prepared using compound NPK 32-10-10 fertilizer and MS medium as the control, with the addition of sucrose according to the treatments, 0.5 ppm BAP, and 6 g/L agar. The pH of the media was adjusted to 5.8 before sterilization using an autoclave for 15 minutes. TSS seeds of the Lokananta variety were sterilized using bactericide, fungicide, 5% chlorox solution, and 70% alcohol, then aseptically planted in a Laminar Air Flow Cabinet (LAFC). The cultures were maintained in a growth room at a temperature of 18–20°C with a photoperiod of 16 hours per day using an automatic timer. Observations were carried out regularly, and contaminated cultures were separated and removed.

Data collection and observations were conducted for 16 weeks after planting, starting one day after planting. Daily observations were performed to record germination percentage, germination time, and the time of micro-shoot emergence. Weekly observations were conducted once a week to record the number of micro-shoots, number of micro-leaves, time of micro-leaf emergence, and time of micro-bulb formation through visual observation from outside the culture bottles. Final observations were carried out at week 16 inside the LAFC by removing the plantlets from the culture bottles and placing them in Petri dishes lined with millimeter block paper to measure plant height, number and length of micro-roots, percentage of micro-bulb formation, and micro-bulb diameter. The collected data were statistically analyzed using Analysis of Variance (ANOVA) with an F-test at the 5% significance level. If significant differences were detected, the analysis was continued using Duncan's Multiple Range Test (DMRT). If the coefficient of variation (CV) value was greater than 30%, data transformation was performed using the formula $\sqrt{(x + 0.5)}$ to reduce the CV value (Cahyani, 2021). If the CV value remained greater than 30% after transformation, the data were analyzed descriptively and presented in the form of histograms.

3. RESULTS AND DISCUSSION

The results showed that there was an interaction between the concentration of compound NPK fertilizer and sucrose supplementation in the culture medium on the variables of

number of roots and number of micro-leaves. The other six variables showed no interaction (Table 1). Sucrose addition had a significant effect on the number of micro-roots and plant height, but had no significant effect on the other six variables. The treatment of compound NPK fertilizer concentration showed a significant effect on all observed variables, except for the percentage of micro-bulb formation, which was not significantly affected.

Table 1. Summary of F-values on the growth and micro-bulb formation of shallot derived from True Shallot Seeds (TSS) under in vitro conditions

	Variable	F-Values			CV (%)
		Compound Fertilizer	Sucrose	Interaction	
1	Germination Percentage	13,14 *	1,08 ns	1,04 ns	22,05
2	Germination Time	11,37 *	91,53 ns	0,53 ns	8,34
3	Number of Micro Shoots	75,51*	1,77 ns	1,44 ns	9,26T
4	Number of Micro Leaves	102,41*	0,22ns	3,40*	17,73T
5	Number of Micro Roots	411,19 *	64,92 *	55,03 *	20,48
6	Micro Root Length	172,40 *	2,41 ns	1,16 ns	11,54T
7	Microbulb Percentage	300,26 ns	7,26 ns	5,11ns	15,50T
8	Microbulb Diameter	84,86 *	1,26 ns	0,50 ns	21,49T
9	Plant Height	483,85 *	7,98 *	3,13 ns	21,71
10	Time to Micro Shoot Emergence	1,66ns	0,85 ns	0,61 ns	38,19T-
11	Time to Micro Leaf Emergence	23,52*	0,10ns	0,06ns	43,07T-
12	Time to Microbulb Emergence	52,69*	0,51ns	0,83ns	36,39T-

*Note: * = significantly affected at the 5% level, ns = not significantly affected at the 5% level. T= data transformed with √(x+0.5), T− = data with KK >30% after transformation.*

3.1 Interaction Effect of Compound NPK Fertilizer Concentration and Sucrose in In Vitro Culture Medium

There was an interaction between the concentration of compound NPK fertilizer and sucrose concentration on the number of micro-roots (Table 2). This interaction indicated different response patterns in the number of micro-roots for each treatment combination. MS medium supplemented with 60 g/L sucrose produced the highest mean number of micro-roots and was significantly different from sucrose concentrations of 30 g/L, 90 g/L, and 120 g/L. Under the 2 g/L compound NPK fertilizer treatment, a sucrose concentration of 60 g/L resulted in the highest mean number of micro-roots and was significantly different from the other sucrose concentrations. A similar pattern was observed under the 4 g/L compound NPK fertilizer treatment; however, all treatment combinations in this group produced a lower number of micro-roots compared to those grown on MS medium. The lower mean number of micro-roots observed in the compound NPK fertilizer media was presumably due to nutrient imbalance, particularly nitrogen, which may inhibit root initiation. This result is consistent with the findings of Utomo and Yunus (2021), who reported that compound NPK fertilizer media produced fewer roots than MS medium due to nitrogen imbalance, leading to nitrogen accumulation in the area where root primordia develop.Overall, the combination of MS medium supplemented with 60 g/L sucrose was the most effective treatment in increasing the number of micro-roots.

Table 2. Interaction of NPK Fertilizer Concentration and Sucrose on Micro-Root Number

Compound	Sucrose Concentration

Fertilizer Concentration	S0 (Sucrose 30g/L)	S1 (Sucrose 60g/L)	S2 (Sucrose 90g/L)	S3 (Sucrose120 g/L)
P0 (Murashige and Skooq)	4,67 c A	12,89 a A	5,70 b A	3,15 d A
P1 (NPK 2 g/L)	0,74 b B	1,48 a B	0,85 b B	1,31 b B
P2 (NPK 4 g/L)	0,56 b B	0,70 a B	0,56 b B	0,67 b B

Note: Numbers followed by the same capital letter and in the same column are not significantly different in the DMRT 5% level follow-up test. Numbers followed by the same lowercase letter and in the same row are not significantly different in the DMRT 5% level follow-up test.

An interaction was observed between the concentration of compound NPK fertilizer and sucrose concentration on the number of micro-leaves (Table 3).

Table 3. Interaction of NPK Fertilizer Concentration and Sucrose on Micro-Leaf Number

Compound Fertilizer Concentration	Sucrose Concentration			
	S0 (Sucrose 30g/L)	S1 (Sucrose 60g/L)	S2 (Sucrose 90g/L)	S3 (Sucrose120 g/L)
P0 (Murashige and Skooq)	1,40 a A	1,99 a A	1,78 a A	1,79 a A
P1 (NPK 2 g/L)	1,05 a A	0,71 a B	0,78 a B	0,71 a B
P2 (NPK 4 g/L)	0,75 a A	0,71 a B	0,71 a B	0,71 a B

Note: Numbers followed by the same capital letter and in the same column are not significantly different in the DMRT 5% level follow-up test. Numbers followed by the same lowercase letter and in the same row are not significantly different in the DMRT 5% level follow-up test.

There was an interaction between the concentration of compound NPK fertilizer and sucrose concentration on the number of micro-leaves (Table 3). This interaction indicated different response patterns in the number of micro-leaves for each treatment combination. In MS medium, a sucrose concentration of 60 g/L resulted in the highest number of micro-leaves and was not significantly different from sucrose concentrations of 30 g/L, 90 g/L, and 120 g/L. These results indicate that increasing sucrose concentration up to a certain level still supports micro-leaf formation. This finding is in line with Rahmawidowati et al. (2022), who reported that increasing sucrose concentration can enhance the availability of carbon and energy sources for explant cells, thereby supporting cell division and differentiation processes involved in micro-leaf formation under in vitro conditions.

In the 2 g/L compound NPK fertilizer treatment, a sucrose concentration of 30 g/L produced the highest number of micro-leaves and was significantly different from sucrose concentrations of 60 g/L, 90 g/L, and 120 g/L, while the latter three concentrations were not significantly different from each other. Similarly, under the 4 g/L compound NPK fertilizer

treatment, a sucrose concentration of 30 g/L also resulted in the highest number of micro-leaves and was significantly different from sucrose concentrations of 60 g/L, 90 g/L, and 120 g/L, which did not differ significantly among themselves. Overall, MS medium produced a higher number of micro-leaves compared to compound NPK fertilizer media at concentrations of 2 g/L and 4 g/L. This result is consistent with the findings of Asharo et al. (2024), who reported that MS medium produced the highest number of leaves compared to compound NPK-based media due to its nitrogen content, which more effectively supports leaf formation. However, the results of this study contrast with those reported by Vallepy et al. (2024), who found that the use of compound NPK fertilizer as a culture medium at concentrations of 3 g/L and 4 g/L resulted in better leaf proliferation. This discrepancy is presumably due to the nitrogen content at those concentrations being sufficient to meet the nutritional requirements of orchids, particularly for chlorophyll formation in leaf tissues, thereby enhancing leaf development.

3.2 Effect of NPK Fertilizer Concentration on Growth and Micro-Bulb Formation of TSS

The treatment of compound NPK fertilizer concentration had a significant effect on the observed variables of plant height, germination time, germination percentage, number of micro-shoots, micro-root length, and micro-bulb diameter.

Table 4. Mean values of compound NPK fertilizer concentration treatments on plant height, germination time, germination percentage, number of shoots, root length, and bulb diameter.

Treatment	Plant height (cm)	Germination time (HST)	Germination percentage (%)	Number of shoots	Root length (cm)	Tuber diameter (mm)
P0 (Murahige and Skooq)	14,73 a	7,59 a	100 a	1,22 a	1,82 a	2,03 a
P1 (Majemuk NPK 2 g/L)	1, 64 b	8,42 ab	81,48 ab	0,95 b	0,87 b	1,10 b
P2 (Majemuk NPK 4 g/L)	0,18 c	9,17 b	55,56 b	0,77 b	0,81 b	0,71 b

Notes: Values followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level.

3.3 The Effect of Sucrose Concentration on the Growth of Shallot Derived from True Shallot Seed (TSS)

The application of various sucrose concentrations in the culture medium of shallots derived from True Shallot Seed (TSS) did not significantly affect the variables of germination percentage, germination time, number of micro shoots, number of micro leaves, micro root length, and bulb diameter. However, plant height was significantly affected by the application of different sucrose concentrations in the culture medium.

Table 5. The mean values of sucrose concentration treatments on the plant height variable.

Treatment	Plant Height (cm)

S0 (Sucrose 30 g/L)	15,73 a
S1 (Sucrose 60 g/L)	14,73 a
S2 (Sucrose 90 g/L)	12,01 b
S3 (Sucrose 120 g/L)	11,31 b

Note: Means followed by the same letter within the same column are not significantly different according to the DMRT at the 5% significance level

The sucrose treatments at 30 g/L and 60 g/L resulted in mean plant height values that were not significantly different from each other, but were significantly different from those at 90 g/L and 120 g/L. Meanwhile, the sucrose treatment at 90 g/L was not significantly different from that at 120 g/L. These results indicate that sucrose concentrations of 30–60 g/L produced the best plant height compared to sucrose concentrations of 90–120 g/L. This condition is consistent with the study by Putri (2024), which reported that culture media supplemented with sucrose in the range of 50–70 g/L were able to enhance shallot plant height under in vitro conditions. Conversely, excessively high sucrose concentrations may induce osmotic stress that inhibits plant tissue growth (Dos Reis and Ayub, 2025; Huh et al., 2016). This finding is also supported by Maharani (2019), who stated that increasing sucrose concentration above the optimum level can suppress plant growth in vitro.

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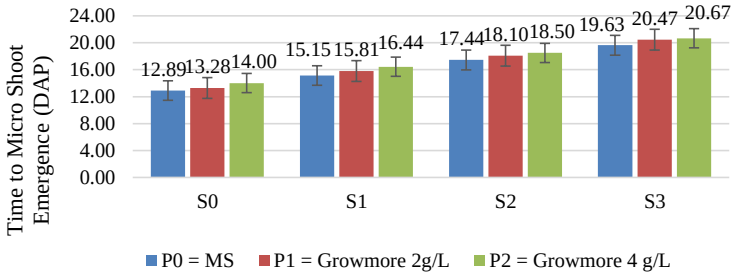
3.4 Descriptive Analysis of Growth and Microbulb Formation of Shallots from True Shallot Seed (TSS)

Descriptive analysis was conducted on the variables of time to shoot emergence, leaf emergence, and microbulb formation because the coefficient of variation (CV) remained above 30% even after data transformation. Therefore, the data were analyzed descriptively and presented in the form of histograms.

3.4.1 Time to Micro Shoot Emergence

The time to micro shoot emergence showed differences among treatments of culture media type and sucrose concentration (Figure 1). MS medium supplemented with 30 g/L sucrose produced a mean time to micro shoot emergence of 12.89 days after planting (DAP). In the compound fertilizer (NPK) medium at a concentration of 2 g/L combined with 30 g/L sucrose, the mean time to micro shoot emergence was 13.28 DAP, whereas the treatment with 4 g/L compound fertilizer and 120 g/L sucrose resulted in the longest mean time to micro shoot emergence, at 20.67 DAP. This pattern indicates that increasing sucrose concentration in the culture medium can inhibit micro shoot formation. These results are consistent with the findings of Triyastuti et al. (2018), who reported that increasing sucrose concentration delayed the time of shoot emergence in chrysanthemum plantlets due to a decrease in the osmotic potential of the culture medium. According to Apriliani et al. (2023), axillary shoots of chrysanthemum grown on media containing higher sucrose concentrations emerged at 2 weeks after planting (WAP), which was one week later than those grown on media with lower sucrose concentrations. In addition to sucrose concentration, delayed micro shoot emergence at high levels of compound fertilizer is presumed to be caused by excess phosphorus in the medium. Excess phosphorus can lead to nutrient imbalance by interfering with the uptake of micronutrients such as Fe and Zn, which play important roles in cell division and differentiation, thereby slowing the initiation of micro shoots (Wahidah and Achmad, 2020). This finding is in line with Isda and Wusqa (2024), who reported that excess phosphorus can result in slower shoot growth.



Ket : S0 = Sucrose 30g/L ; S1 = Sucrose 60 g/L ;
S2 = Sucrose 90g/L ; S3 = Sucrose 120 g/L

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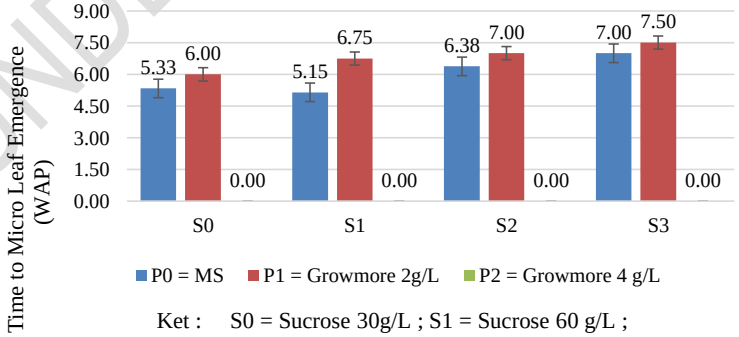
Figure 1. Effect of compound fertilizer and sucrose concentrations on the time to micro shoot emergence of shallots under in vitro conditions.

3.4.2 Time to Micro Leaf Emergence

The time to micro leaf emergence differed among treatments of culture media type and sucrose concentration (Figure 2). MS medium supplemented with 60 g/L sucrose resulted in a mean time to micro leaf emergence of 5.15 weeks after planting (WAP). In the compound fertilizer medium at a concentration of 2 g/L combined with 30 g/L sucrose, the mean time to micro leaf emergence was 6.00 WAP, whereas the treatment with 2 g/L compound fertilizer and 120 g/L sucrose showed a mean time of 7.50 WAP. This pattern indicates that the time to micro leaf emergence tends to increase with increasing sucrose concentration in the culture medium. These results are consistent with the findings of Hapsari and Saptadi (2018), who reported that in culture media containing 90 g/L and 120 g/L sucrose, the time to leaf emergence of garlic explants tended to be longer than in media with sucrose concentrations of 30 g/L and 60 g/L. In addition to sucrose concentration, leaf formation was also inhibited by high concentrations of compound fertilizer (NPK). In the medium containing 4 g/L compound fertilizer, none of the treatments showed leaf emergence until the end of the observation period. This finding is in line with Sumihar et al. (2021), who reported that leaves of Raja Bulu banana explants had not formed at 5 WAP in culture media containing NPK compound fertilizer 32–10–10, whereas leaf formation in MS medium began at 5 WAP. This condition is presumably caused by osmotic stress in the medium due to excessive nitrogen, which can suppress leaf growth, induce toxicity in the form of necrosis, and interfere with water uptake (Hardianti and Soetopo, 2019).



Ket : S0 = Sucrose 30g/L ; S1 = Sucrose 60 g/L ;
S2 = Sucrose 90g/L ; S3 = Sucrose 120 g/L

Figure 2. Effects of compound fertilizer and sucrose concentrations on micro leaf emergence time of shallots in vitro.

3.4.3 Time to Microbulb Emergence

The time to microbulb formation showed clear differences among culture media and sucrose concentration treatments (Figure 3). In MS medium supplemented with 60 g/L sucrose, microbulb formation occurred at 7.71 weeks after planting (WAP). This result is consistent with the statement of Hapsari and Saptadi (2018), who reported that a sucrose concentration of 60 g/L has the potential to induce microbulb formation. In the compound fertilizer medium, microbulbs were formed only at a concentration of 2 g/L combined with sucrose concentrations of 30 and 60 g/L. This condition is presumably due to the addition of compound fertilizer increasing the nutrient content of the culture medium. Excessive nutrient availability is unfavorable for plant growth. Excess nitrogen can inhibit the uptake of other nutrients such as potassium, while excess phosphorus can slow growth, particularly shoot growth, and excessive potassium may cause toxicity leading to plant damage or death (Isda and Wusqa, 2024).

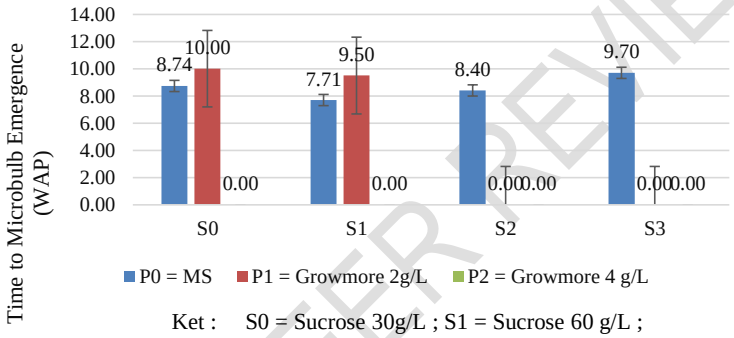


Figure 3. Effect of compound fertilizer and sucrose concentrations on the time to microbulb emergence of shallots under in vitro conditions.

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4. CONCLUSION

The combination of MS medium supplemented with 60 g/L sucrose produced the highest number of leaves and roots. MS medium resulted in the highest mean values for the variables of germination time, germination percentage, plant height, number of shoots, root length, and bulb diameter. However, the compound fertilizer (NPK) medium at a concentration of 2 g/L was only able to match MS medium in terms of germination percentage and germination time. Therefore, MS medium supplemented with 60 g/L sucrose was identified as the best combination to support growth and microbulb formation of shallots derived from True Shallot Seed (TSS) under in vitro conditions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors hereby declare that ChatGPT (OpenAI) was used solely to assist with language editing, sentence structure improvement, and translation to enhance the clarity and readability of the manuscript. All scientific content, data analysis, interpretation of results, and final conclusions were thoroughly reviewed, verified, and fully approved by the authors. The use of artificial intelligence did not affect the scientific integrity or originality of this work.

التعليق **[M8]:** References and the citation inside the text should be as per Journal's guidelines.

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