Quagga
Jurnal Pendidikan dan Biologi

p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 16, No. 1, 2024, pp 94 - 101
Available online https://journal.fkip.uniku.ac.id/quagga/index
DOI: 10.25134/quagga.v16i1.91

The Effect of *Thiamine HCL* Concentration Variation on the Seedling of *Phalaenopsis* Var. *Happy Valentine*

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APA Citation: Handini., Dzakiy, M. A., & Hartadiyati, E. (2024). The Effect of Thiamine HCL Concentration Variation on the Seedling of Phalaenopsis Var. Happy Valentine. *Quagga: Journal of Education and Biology*, 16(1), 94-101. DOI: 10.25134/quagga.v16i1.91.

Received: 16-11-2023 Accepted: 07-12-2023 Published: 01-01-2024

***Abstract**: Phalaenopsis var. Happy Valentine orchid is a plant with a growth and flowering process of approximately 1-2 years. Generally, orchids can bloom in about 9-12 months. Optimal concentrations of Thiamine HCl (vitamin B_1) are required to support the vegetative growth of Phalaenopsis var. Happy orchids. This research aims to determine the effect and the best concentration of vitamin B_1 among 1ml, 2ml, and 3ml on the vegetative growth, including leaf span, root quantity, leaf width, and length of young leaves of Phalaenopsis var. Happy Valentine seedlings. The method used was a Completely Randomized Design (CRD), with treatment factors being the concentration of vitamin B_1 and Growmore fertilizer, comprising four treatment levels and six replications, namely V0 = 2gr/L Growmore fertilizer, V1 = 2gr/L Growmore fertilizer + 1ml B_1, V2 = 2gr/L Growmore fertilizer + 2ml B_1, dan V3 = 2gr/L Growmore fertilizer + 3ml B_1. This research used 24 Phalaenopsis var. Happy Valentine orchid seedlings as research samples. The data were analyzed using ANOVA with a significance level 0.05 and subsequently subjected to Duncan's test. The results indicate that vitamin B_1 significantly affects leaf span, length of young leaves, and the quantity of roots in Phalaenopsis var. Happy Valentine.*

***Keywords:** Concentration; Phalaenopsis; Thiamine HCl*

1. INTRODUCTION

Biodiversity in Indonesia is significantly high (Alang et al., 2022). One of these is the orchid, particularly the *Phalaenopsis* orchid, which has held economic significance (Yang et al., 2021), especially on an industrial scale in recent decades (Chao et al., 2018). This is due to its decorative value and high aesthetic diversity (Pudji Restanto et al., 2021). Moreover, *Phalaenopsis* orchids have various unique flower forms (Lin et al., 2016). As a result, *Phalaenopsis* orchids have become some of the most sought-after varieties. However, some orchid species are on the verge of extinction due to a dramatic decline (Shao et al., 2022). Orchids from the *Phalaenopsis* genus have an extended blooming period (Tong et al., 2020).

Phalaenopsis can bloom two or three times a year, with each blooming period lasting around 1-2 months. Only after two to three years do *Phalaenopsis* orchids begin to flower (Rakhmawati Dewi, 2019). Therefore, with proper care and attention, such as fertilization and a conducive growing environment, orchid plants can flourish and produce more flowers. (Sukartini et al., 2014).

Essentially, the commonly used vitamin is Thiamine (B_1) because *Thiamin* (B_1) functions in carbohydrate metabolism, playing a role in converting glucose or sugar into energy (Kusmiadi et al., 2023). This makes Thiamine applicable to plants, including *Phalaenopsis* orchids, requiring ample growth energy. Additionally, Thiamine is also needed to

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DOI: 10.25134/quagga.v16i1.91

synthesize sugar and amino acids, which are the basic building blocks for plant growth and development, helping plants overcome growth stress and build new tissues. Similar to plants in general, *Phalaenopsis* orchids require fertilizers to promote their vegetative growth (Surtinah, 2013.).

Seedling *Phalaenopsis* orchids are often chosen for research because these orchids are initially planted in culture bottles for one year. Afterward, the plants are ready to be transferred to a more extreme environment or acclimatized. At the age of 8 months post-acclimatization, they are referred to as seedlings and are considered to easily adapt to the environment, provided they are given proper treatment. Therefore, fertilizer is necessary at this stage to promote the growth of *Phalaenopsis* orchid seedlings (Surtinah, 2013). The seedling phase marks the transition from seedling to adolescence, making it a phase where vegetative growth can be observed rapidly. Vegetative growth parameters considered include leaves and roots due to the growth pattern of the monopodial type of *Phalaenopsis* orchid (Arobaya, 2022); therefore, measuring it specifically is challenging since only the pseudostem grows. Meanwhile, the leaves of *Phalaenopsis* orchids are attached to tree bark in a riding position (Erwindah et al., 2022). The plentiful and branching root structure makes counting the number of roots in *Phalaenopsis* orchids easier. Having numerous and long roots can contribute to better growth. The more roots a plant has, the more efficient it is in absorbing water and enhancing nutrient value for plant growth (Liu et al., 2019). Roots also serve as a storage place for energy in the form of carbohydrates (Risdiana et al., 2023).

Fertilization for *Phalaenopsis* orchids occurs in the late afternoon because the transpiration process occurs most rapidly during this time. The orchid stomata can open, and environmental factors accelerate this process. Furthermore, evening fertilization promotes faster plant growth as it accumulates more nitrogen compared to morning fertilization. Therefore, this research aims to determine the effect and optimal treatment factor of vitamin B_1 concentration for the vegetative growth of *Phalaenopsis* var. *Happy Valentine* is measured based on leaf length, leaf width, root quantity, and length of young leaves.

2. RESEARCH METHODOLOGY

The study was conducted at CV. Candi Orchid, Jl. Bukit Unggul Raya No.17, Bendan Ngisor, Semarang City, during the months of May to July 2023. This research employed an experimental method to examine the effect and optimal concentration of three treatments of vitamin B_1 (Anisa et al., 2018). Data were collected through direct observation and recording, involving several stages such as equipment sterilization (Annisa et al., 2020) using 70% alcohol for 30 seconds (Muna et al., 2022), followed by material preparation, planting media preparation, treatment solution dilution preparation, and spraying stages. There were four groups, each with six samples of *Phalaenopsis* var. *Happy Valentine* orchids. Each sample had four leaves, six roots and was an 8-month-old seedling post-acclimatization. The orchids were planted in 1.7-inch soft pots using a planting medium with 150 grams of kadaka root.

The preparation of the Growmore fertilizer solution for the treatment involved weighing 2 grams of Growmore fertilizer powder and adding it to 1000 ml of regular water in a measuring glass, then stirring thoroughly. A 4-liter solution of Growmore fertilizer was prepared for the control group, and the mixture of 3 concentrations of Thiamine HCl. In the preparation of the vitamin B_1 solution using a dosage of 1 ppm, the method involved preparing vitamin B_1 and distilled water in a ratio of 1:100. In a measuring glass, filled 100 ml of distilled water (aquades), then injected 1 ml of vitamin B_1 using a syringe and stirred slowly. Pour the vitamin B_1 solution into a glass container, seal it tightly with aluminum foil, relayered it with plastic, and secure it with a rubber band. The resulting vitamin B_1 solution was placed in the refrigerator and observed for 24 hours to ensure no contamination. After one day without contamination, the solution could be used on plants and applied according to the planned research concentrations. In the V0 group (Control), treatment involved using only 2 grams/L Growmore fertilizer.

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After that, in group V1, 2 grams/L of Growmore fertilizer + 1 ml/L of B_1 concentration were used. Group V2 used 2 grams/L of Growmore fertilizer + 2 ml/L of B_1 concentration, and group V3 used 2 grams/L of Growmore fertilizer + 3 ml/L of B_1 concentration. The vitamins were given twice a week, between 3:00 PM – 5:00 PM, for a total of 12 weeks. Research data were collected from measurements of leaf span (cm), length of young leaves (cm), leaf width (cm), and the number of roots. The data analysis technique used was one-way ANOVA with a significance level of 0.05, followed by Duncan's test. SPSS 27 was used to process the data.

3. RESULTS AND DISCUSSION

Research on orchids using vitamin B_1 has been extensively conducted (Gusti et al., 2013). However, this study is specific as it focuses on seedling orchids of *Phalaenopsis* var. *Happy Valentine* to generate continuous data compared to previous research (Sebastian, 2022). The study used parameters such as leaf span, leaf width, root quantity, and young leaf length, which were modified from previous research parameters. Thiamine (B_1) functions in carbohydrate metabolism, playing a role in converting glucose or sugar into energy (Kusmiadi et al., 2023) that can be utilized by plants because *Phalaenopsis* orchids require a considerable amount of energy to grow, and vitamin B_1 helps enhance this process. Orchids cannot obtain the necessary nutrients from the environment, thus requiring a steady supply of macro and micro components (Dwi Agustiar et al., 2020). This is why vitamin B_1 is often chosen for *Phalaenopsis* orchids. As stated by Aini et al. (2016) Thiamine (B_1) supplementation in orchids has proven to stimulate cell division, accelerating growth by enhancing hormonal activity in plant tissues. Consistent with the findings of Latif et al. (2020), the application of vitamin B_1 to *Phalaenopsis* orchids will increase leaf production and the number of new roots produced because vitamin B_1 stimulates cell proliferation in root meristems.

The research results on the leaf span of *Phalaenopsis* orchids are presented in Table 2.

Table 1. Anova Results for Leaf Span

	df	Mean	F	Sig
Between Groups	3	27.923	3.382	0.038
Within Groups	20	8.256		
Total	23			

sig < 0.05: There is a significant effect of the application of vitamin B_1 treatment.

Table 2. Duncan's Test Results for Leaf Span

treatment	V0	V1	V2	V3
Leaf Span (cm)	3,68 [a]	6,10 [ab]	7,92 [b]	8,48 [b]

[a,b] : Similar letter notations indicate no significant difference at the 5% Duncan test level.

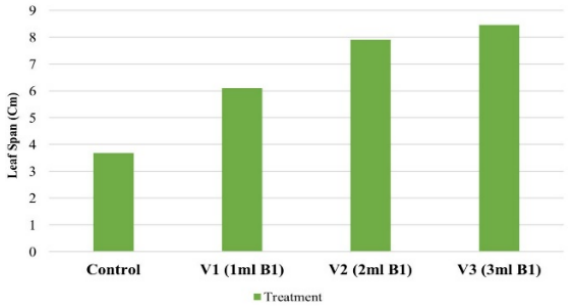

Figure 1. Average Leaf Span Growth Chart

The best concentration for leaf span can be achieved using a 2 ml/L concentration. The optimal concentration can be selected as 2 ml because, economically speaking, both the 2 ml and 3 ml concentrations have a similar effect on the growth of the leaf span of *Phalaenopsis* orchids. Therefore, a concentration of 2 ml of vitamin B_1 can be used as it requires fewer materials, resulting in lower expenses and supporting the efforts of farmers.

The absorption process of vitamin B_1 can affect the growth of the leaf span through several stages. Roots absorb nutrients through ions through osmosis (Amir, 2016). These roots have a lower nutrient concentration than the surrounding soil solution (Gusti et al., 2013). Water enters the root cells passively through the cell membrane due to concentration differences, carrying mineral ions or dissolved nutrients for plant growth (Yahya, 2015). The difference in substance concentration across the cell membrane causes osmotic pressure, leading to the movement of molecules from an area of high concentration to low concentration (Musliman & Damayanti, 2023). This process occurs through a semi-

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DOI: 10.25134/quagga.v16i1.91

permeable cell membrane, which is permeable to water molecules that have sizes corresponding to the membrane pores (Musliman & Damayanti, 2023). The movement of water and dissolved nutrients from the roots to the leaves is referred to as the water transport system in plants. The concentration of water at the root tip increases, causing a difference in concentration between the cells at the root tip and those above, resulting in root pressure. This pressure helps push water and nutrients upward for use in the plant's vegetative growth (Sobari, 2020). Therefore, vitamin B_1 can affect the leaf span growth of *Phalaenopsis* orchids.

Furthermore, measurements of the length of young leaves, as indicated in Table 4, show no significant differences. However, between the control group and all concentrations of vitamin B_1 treatments, there are statistically significant effects, as indicated by the differing notations. Based on the analysis results, the optimal concentration for the length of young leaves is 3ml/L. This aligns with the findings of Latif et al. (2020), who suggested that a concentration of 3ml/L of Vitamin B_1 (Thiamin HCl) is most favorable for orchid growth. Duncan's test results indicate that Thiamin is a critical factor in accelerating cell division (Raodah Garuda et al., 2015).

Table 3. Anova Results for Young Leaf Length

	df	Mean	F	Sig
Between Groups	3	26.682	3.224	.044
Within Groups	20	8.276		
Total	23			

sig < 0.05: There is an effect of the application of vitamin B_1 treatment.

Table 4. Duncan's Test Results for Young Leaf Length

treatment	V0	V1	V2	V3
Young Leaf Length (cm)	4.50 [a]	6.45 [ab]	6.78 [ab]	9.62 [b]

[a,b] : Similar letter notations indicate no significant difference at the 5% Duncan test level

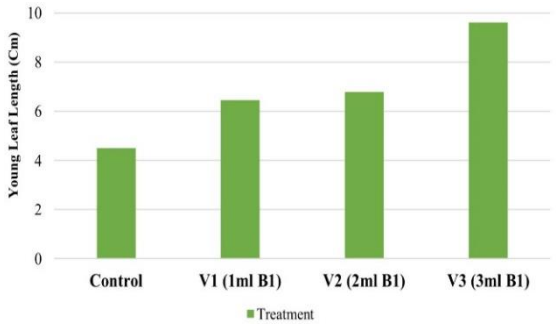

Figure 2. Average Young Leaf Length Growth Chart

Adding 3ml of Thiamin HCl is the optimal concentration for leaf length growth. As observed from the results of young leaf length, the treatment with a concentration of 3ml Vitamin B_1 (Thiamin HCl) indicates the best leaf growth, although the difference is not significant. It can be affirmed that the application of Thiamin HCl stimulates the activity of hormones in plant tissues, facilitating cell division, enlargement, and the formation of new cells (Yustitia R. Inung, 2017). Because vitamin B_1 can accelerate cell division, it stimulates the formation of new leaf buds at the apex of the shoot (Clarah et al., 2017). Leaf growth will also increase compared to using regular fertilizers without the addition of vitamin B_1. In this study, the addition of vitamin B_1 resulted in a noticeable increase in leaf growth, as evidenced by the faster growth of young leaves compared to the control treatment that only used fertilizer. The primary function of Thiamin in plants is as a cofactor (Amalia et al., 2015) in various enzymatic reactions involved in carbohydrate and energy metabolism.

In the observed number of new roots, the produced vitamin B_1 has an effect, and the concentration of 3ml/L is the optimal concentration, as seen in Table 6.

Table 5. Anova Results for Number of Roots

	df	Mean	F	Sig
Between Groups	3	5.000	3.226	.044
Within Groups	20	1.550		
Total	23			

sig < 0.05: There is an effect of the application of vitamin B_1 treatment.

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Table 6. Duncan's Test Results for Number of Roots

treatment	V0	V1	V2	V3
Number of Roots	7,00 [a]	7,67 [ab]	8,17 [ab]	9,17 [b]

[a,b] : Similar letter notations indicate no significant difference at the 5% Duncan test level.

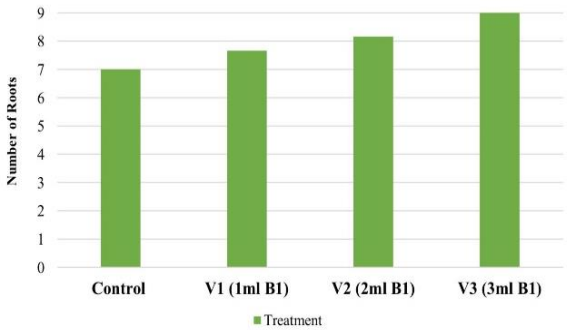

Figure 3. Average Root Number Chart

There is a significant difference between the control (V0) and vitamin B_1; however, the treatment concentration of 3ml (V3) shows more growth even compared to the 1ml and 2ml concentrations, where the number of roots is not as significant. The application of vitamin B_1 (Thiamin HCl) is an essential component because it can accelerate cell division in the root meristem. This is supported by research (Raodah Garuda et al., 2015). There is a significant difference between the control (V0) and vitamin B_1; however, the treatment concentration of 3ml (V3) shows more growth even compared to the 1ml and 2ml concentrations, where the number of roots is not as significant. The application of vitamin B_1 (Thiamin HCl) is an essential component because it can accelerate cell division in the root meristem. This is supported by Hapsari & Lestari (2016) research, which found that Thiamin is one form of vitamin B_1 capable of enhancing cell division in the roots, resulting in faster growth, and branching of the roots. Active cell growth typically requires vitamin B_1, which acts as a catalyst or stimulant in metabolism.

In leaf width, vitamin B_1 does not affect *Phalaenopsis* orchid seedlings. This is indicated in Table 7.

Table 7. Anova Results for Leaf Width

	df	Mean	F	Sig
Between Groups	3	.020	.247	.863
Within Groups	20	.83		
Total	23			

Sig < 0.05: There is an effect of the application of vitamin B_1 treatment.

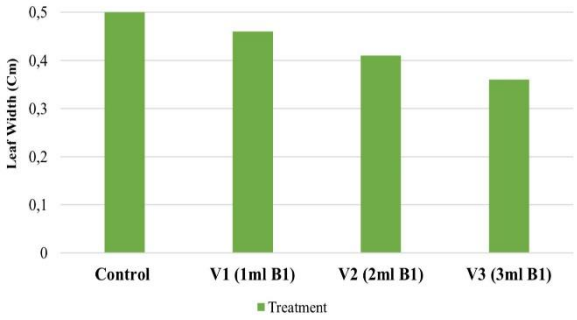

Figure 4. Average Leaf Width Growth Chart

The obtained sig. (0.863) > 0.05 indicates no significant effect of changes in vitamin B_1 (Thiamin HCl) concentration on the leaf width of *Phalaenopsis*. Therefore, no further tests are needed.

Regarding leaf width, vitamin B_1 (Thiamin HCl) has not been able to accelerate leaf expansion. The best leaf width is obtained in the control treatment (V0), which uses growmore fertilizer at a concentration of 2gr/L. This is because fertilizers are generally designed to provide the complete nutrition needed by plants. Fertilizers contain a mixture of nutrients such as nitrogen, phosphorus, potassium, and micro elements required for the overall growth of plants, including leaves; as stated by Dwi Purnomo et al. (2015), the content in fertilizer will compensate for nitrogen deficiencies, resulting in broader and greener leaves. Using fertilizer is more effective in supporting overall plant growth, including widening leaf width. Meanwhile, vitamin B_1 only plays a role in carbohydrate metabolism, with no direct effect on the widening of leaves. The lack of effect of vitamin B_1 on leaf width is also caused by nutrient factors contributing to leaf span and the length of young leaves, causing a decrease in leaf width. Another reason is the morphology of *Phalaenopsis* orchid leaves, which are elongated

p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 16, No. 1, 2024, pp 94 - 101
Available online https://journal.fkip.uniku.ac.id/quagga/index
DOI: 10.25134/quagga.v16i1.91

and tapering, directing leaf growth more towards elongation rather than width (Ayuningtyas et al., 2020).

4. CONCLUSION

Vitamin B_1 (Thiamin HCl) has an effect in supporting the vegetative growth of *Phalaenopsis* var. *Happy Valentine* orchid seedlings, specifically in leaf span, length of young leaves, and the number of roots, but it does not affect leaf width. The optimal concentration of 2 ml/L was found to significantly affect the expansion of the leaf span. Similarly, vitamin B_1 indicates an effect on the growth of young leaf length and the number of roots at a concentration of 3 ml/L. Consequently, *Phalaenopsis* orchid seedlings, which typically exhibit slow growth, can be supported to achieve faster vegetative growth by utilizing vitamin B_1.

5. ACKNOWLEDGEMENTS

The researcher expresses gratitude for the partner CV. Candi Orchid, Mr. Sudibyo Ari Prabowo, and Mrs. Eni Asriati for sharing their expertise and dedicating their time to assist in completing this research.

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p-ISSN 1907-3089, e-ISSN 2615-5869
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Jurnal Pendidikan dan Biologi

p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 16, No. 1, 2024, pp 94 - 101
Available online https://journal.fkip.uniku.ac.id/quagga/index
DOI: 10.25134/quagga.v16i1.91

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