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Concentration Test for Ferrous Sulfate ($FeSO_4$) and Thiamin in *Murashige* and *Skoog* Medium on The Orchid Sub-Culture Dendrobium SP By In-Vitro

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Abstract: The Dendrobium orchid is one type with special features and many variations. This study aims to determine the administration of various concentrations of ferrous sulfate ($FeSO_4$) and Thiamin to Dendrobium sp orchid explants on Murashige And Skoog media. The design used in this study was a Factorial Completely Randomized Design (CRD) consisting of 2 treatment levels (F = $FeSO_4$ and T = Thiamin) with three replications. Namely: F_0 (Without $FeSO_4$), F_1 ($FeSO_4$ 26.8 mg/l), F_2 ($FeSO_4$ 27.8 mg/l), F_3 ($FeSO_4$ 28.8 mg/l), and T_0 (Without Thiamin), T_1 (Thiamin 0.1 mg/l), T_2 (Thiamin 0.2 mg/l) l), T_3 (Thiamin 0.3 mg/l). Based on the study results, by giving various concentrations of ferrous sulfate ($FeSO_4$) had a single effect on the shoots quantity parameters with the best treatment found in treatment with an average shoots quantity (.03 ea), F3 for shoot height parameters (1.37 cm), for the total leaves (6.14), for the roots quantity (5.17), and root length were found in treatment with an average of 1.29 cm on the Dendrobium sp. Orchid explants. For the treatment of various concentrations of Thiamin, the single effect on the number of shoots with the best treatment was found at an average (3.44 ea), for the shoot height parameter (0.98 cm), and for the leaves quantity (6.14 ea), for the root length parameter (1.28 cm). The interaction significantly affected the observed parameters, namely the number of shoots with treatment (giving $FeSO_4$ 28.8 mg/l and Thiamin 0.3 mg/l) with an average shoot quantity of 1.71 (ea).

Keywords: Dendrobium sp; $FeSO_4$; In-vitro; Thiamin.

Introduction

Orchids are ornamental plants with many enthusiasts in various provinces and regions in Indonesia (Kurniawan et al., 2021; Pammai et al., 2022). Dendrobium orchids are one of the most popular orchid genera in the community. This type of orchid is in great demand as a potted plant, cut flower, or garden ornament with high economic value according to the type, shape, flower color, flower character, and species scarcity (Mekapogu et al., 2022).

Market demand for orchids tends to increase, but the development of orchid production in Indonesia is still relatively slow due to the lack of availability of quality seeds, inefficient cultivation, and poor post-harvest handling (Widiastoety, 2001). Dendrobium is one type of orchid that occupies the top position in the order of orchid market trends (Novianto, 2012). Dendrobium has several features: easy to plant, continuous flowering, perfect flower shape, varied flower colors, flexible stems that make it easy to assemble, flower petals do not fall off, and long-lasting flower freshness (Safitri, 2021). With the increasing market demand for orchids, quality seeds are needed in large quantities and quickly.

Dendrobium sp. orchids is an epiphytic orchid that stores food and water reserves in stems, leaves, and roots. The longer the size of the orchid stem, the more food reserves are stored and the ability to absorb water and nutrients more and more (Prasetyo, 2019).

In-vitro plant culture is a cultivation technique that is widely practiced by scientists and researchers, especially in agriculture—considering that agricultural land in Indonesia is decreasing every year due to

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changes in land use. Consumer demand for the willingness of orchids to be used as ornamental plants is a medium-sized business opportunity that needs to be increased. The orchid seed business can improve the community's economy, from ornamental plants to orchid flowers, which is very profitable. For this reason, a more effective alternative method of propagation is needed, namely through in vitro culture or tissue culture. Crossed orchids have a large diversity of properties, which provides an opportunity to select the best progeny to be propagated en masse with in-vitro culture techniques (Vo et al., 2019)

In-vitro culture techniques are used to obtain large quantities of orchid seeds relatively quickly. Naturally, orchids are often difficult to germinate due to unfavorable environmental factors. Therefore, implementing in-vitro seedling techniques can provide benefits in terms of saving space, time, energy, and money. Commercial in-vitro techniques have produced large quantities of plants in a short time. Orchid is a type of plant that has been propagated by in-vitro culture. Media commonly used in the in-vitro culture of orchids include Vacint and Went (VW) media, Knudson C (KC) media, and Murashige and Skoog (MS) media.

Orchid in-vitro culture media can be simplified by using materials that are cheaper and easier to obtain. (Yusnita, 2016) suggests tissue culture techniques are plant propagation techniques by growing plant parts, whether in the form of cells, tissues, or organs, under aseptically in vitro. This technique is characterized by aseptic culture conditions, artificial culture media with complete nutritional content and ZPT (growth regulators), and culture room conditions with controlled temperature and lighting. The success of plant propagation with tissue culture is largely determined by the media used, one of which is the Murashige and Skoog (MS) media.

Murashige and Skoog (MS) is a widely used medium because it contains complete macro and micronutrients that can be used for various plant species (Mardin, 2002). Tissue culture techniques generally have obstacles from the root induction process. A deficiency of sulfate in the media causes this. Sulfate given to the media is usually in the form of $FeSO_4$. The function of $FeSO_4$ is to provide growth of roots, leaves, and photosynthesis.

Fe or ferrous sulfate is a chemical compound with the formula $FeSO_4$. The blue-green heptahydrate Fe or ferrous sulfate is the most common form of this material and has been known since ancient times as copperas and green vitriol. All ferrous sulfates dissolve in water and form the water metal complex $Fe(H_2O)_6^{2+}$. Iron sulfate has an octahedral molecular geometry and is paramagnetic. One of the micronutrients needed by plants is Fe which plays a role in the formation of lignin and chlorophyll (Singhal et al., 2022). The element $FeSO_4$ (iron) plays a role in forming proteins as a catalyst for forming chlorophyll, an electron carrier in photosynthesis and respiration (Mamik, 2016).

Nutrient content apart from macro and micronutrients is also influenced by vitamins. Vitamins play a role in the growth process as a catalyst in metabolism (Widiastoety et al., 2009). One of the vitamins commonly used in tissue culture is Thiamin (vitamin B1). Vitamin B1 is needed as a catalyst and co-enzyme function (Munir & Jariah, 2016). Thiamin (vitamin B1) in orchids can increase the activity of hormones present in tissues so that they can accelerate the division of new cells. Thiamin can induce the highest dendrobium orchid seeds than myacin and pyridoxine (Amalia et al., 2013).

Method

Time and Place

This research has been carried out in the tissue culture laboratory. UPT seeding and seed certification Service for Food Crops and Horticulture Riau Province, Jalan Kaharudin Nasution No. 33 Simpang Tiga Village, Bukit Raya District, Pekanbaru City. This research was carried out for four months, from October 2021 to January 2022.

Tools and Materials

The tools used in this study were laminar air flow cabinets, measuring cups, beakers, petridishes, pipettes, autoclaves, analytical balances, Erlenmayer, magnetic stirrers, glass stirrers, tweezers, scarpels, spirit lamps, hand sprayers, pH meters, knives, culture bottles, gas burners, measuring flasks, test tubes, plastic rubber, scissors, aluminum foil, stationery and washing equipment for supporting activities in tissue culture research.

The materials used in this study were *Dendrobium* sp. Orchid explants, sucrose and Thiamin, MS media, alcohol, agar powder, sterile distilled water, detergent, twin, fungicide, rubber bands, label paper, and other materials that support the manufacture of tissue culture planting media.

The tools were sterilized in an autoclave wrapped in aluminum foil at 121^{o}C for 1 hour at a pressure of 17.5 psi. Aquades were sterilized using an Erlenmeyer containing 1000 ml of Aquades covered with aluminum foil and plastic and put in an autoclave for 1 hour at 121oC with a pressure of 17.5 psi.

The inoculation room is sterilized by spraying with 90% alcohol on the inside of the laminated air flow cabinet, followed by turning on the neon lamp and blower. Labeling was done before giving a treatment by attaching it to each bottle.

The culture medium used was modified Murashige and Skoog (MS) media consisting of sucrose, vitamins,

agar, ZPT (NAA and BAP), macro elements (KNO_3, NH_4NO_3, $MgSO_47H_2O$, KH_2PO_4), and microelements (MnSO4). $4H_2O$, $ZnSO_47H_2O$, $H3BO_4$, Kl, $Na_2MO_42H_2O$, $CuCO_45H_2O$, $FeSO_4$ and ($CaCl_26H_2O$).

$FeSO_4$ solution and Thiamin solution were dissolved with 100 ml of distilled water and then added to a solution volume of 1,000 ml. After the complete solution, the stock solution is stored in the refrigerator.

Planting is done in a laminar airflow cabinet (LAFC), which is sterilized by turning on a UV (ultraviolet) lamp for one hour and spraying it with 90% alcohol before use. Explants of *Dendrobium* SP orchids in petri dishes were taken using tweezers and planted in culture bottle media. The mouth of the bottle is burned with a bunsen lamp slowly while rotating it.

The design used in this study was a Factorial Completely Randomized Design (CRD) consisting of 2 treatment levels (F = $FeSO_4$ and T = Thiamin) with three replications. Namely: F_0 (Without $FeSO_4$), F1 ($FeSO_4$ 26.8 mg/l), F2 ($FeSO_4$ 27.8 mg/l), F3 ($FeSO_4$ 28.8 mg/l), and T0 (Without Thiamin), T1 (Thiamin 0.1 mg/l), T2 (Thiamin 0.2 mg/l) l), T3 (Thiamin 0.3 mg/l).

Result and Discussion

Number of shoots (Fruit)

Based on the results of observations on the parameter of the number of shoots of Dendrobium sp orchid explants, after analysis showed that the treatment of ferosulfate ($FeSO_4$) and Thiamin alone had a significant effect on the number of shoots of Dendrobium sp orchid explants, and on an interaction basis the administration of ferrosulfate ($FeSO_4$). The results of the honest significant difference test (BNJ) at the 5% level can be seen in table 1.

Table 1. Average number of shoots of Dendrobium sp orchid explants treated with ferrous sulfate ($FeSO_4$) and thiamin on MS media (fruit)

Factor F	Factor T				Average FT
	Thiamin (0 m/l)	Thiamin (0.1 mg/l)	Thiamin (0.2 mg/l)	Thiamin (0.3 mg/l)	
Control	3.22 bc	3.22 bc	3.11 bc	3.67 c	3.31 ab
$FeSO_4$ 26.8 mg/l	3.11 bc	3.33 bc	3.56 b	2.33 c	3.08 b
$FeSO_4$ 27.8 mg/l	2.33 c	4.22 ab	4.44 a	3.44 bc	3.61 a
$FeSO_4$ 28.8 mg/l	4.33 a	2.67 c	2.67 c	2.44 c	3.03 b
Average T	3.25 ab	3.36 a	3.44 a	2.97 b	
KK = 8.90 %		BNJ F = 0.32	BNJ T = 0.32		FT = 0.86

Note: the numbers in the rows and columns followed by the same lowercase letters are not significantly different according to the Significant Difference Advanced Test (BNJ) at the 5% level.

The data in table 1 can be seen that the administration of Fero Sulfate ($FeSO_4$) with the best treatment was found in the administration of $FeSO_4$ 27.8 mg/l MS media, namely with the number of shoots of 3.61, the results of the further test were significantly different (BNJ) at the 5% level showed that this treatment was significantly different from the F1 treatment (3.08 fruit) and F_3 (3.03 fruit), but not significantly different from the F_0 treatment (3.31 fruit).

Concentration of $FeSO_4$ (27.8 mg/l) gave the best results compared to other concentrations of treatment. This was because treatment with a concentration of $FeSO_4$ (27.8 mg/l MS media) was the right concentration to be given to explants. Orchid Dendrobium sp. Plant cells and tissues in culture media lack autotrophic ability and therefore, require external $FeSO_4$. The addition of external sources of iron and sulfur to the media increased cell proliferation and regeneration of green shoots. optimal concentration of $FeSO_4$ in the media should be sufficient to meet basic energy requirements for cell division, differentiation and not cause negative osmotic effects on shoot formation. This shows that the concentration of $FeSO_4$ is one of the factors that control the induction and growth of shoots. Shoot growth is also affected by differences in sucrose concentration (Gibson & Fuller, 2000).

The MS media used for the nutrient element $FeSO_4$ as much as 28.8 mg/l, giving $FeSO_4$ to the basic media produced a number of shoots of 8.66 strands of Dendrobium sp. Whereas in this study the administration of $FeSO_4$ as much as 27.8 mg/l resulted in a total of 3.61 shoots. This is due to the different concentration of $FeSO_4$ given, the resulting response is also different.

Based on table 1, it shows that giving Thiamin has a significant effect on the parameters of the number of shoots of Dendrobium sp orchid explants with the best treatment found in the treatment of giving Thiamin as much as 0.2 into MS media, namely 3.44 pieces, from the results of the further difference test honest significant difference (BNJ) on The 5% level indicated that the treatment was significantly different from giving Thiamin 0.3 mg/l, namely 2.97 fruit, but not significantly different from the treatment with Thiamin 0.1 mg/l, namely 3.36 fruit and without Thiamin, namely 3.25 fruit.

Thiamin concentration (0.1 mg/l) gave the best results compared to the concentration given at T1, T0 and T3 treatments. This was because the T2 treatment

with Thiamin concentration (0.1 mg/l MS medium) was the right concentration to be given to Dendrobium sp. Giving Thiamin in small amounts is able to meet the needs of plants, even though each plant already has an endogenous ZPT but it needs to be given more vitamins so that the needs of the plants can be fulfilled properly (Winarso, 2015). This is Matter of Thiamin at this concentration is most suitable for the needs of Dendrobium sp orchid plant explants on MS media. According to Amalia et al. (2013) Thiamin in orchids can increase the activity of hormones found in tissues so that they can accelerate the division of new cells. Thiamin can induce the highest dendrobium orchid seeds than myacin and peridoxin (Amalia et al., 2013).

The conclusions that the administration of 1 mg/l thiamin into MS media had a significant effect on the growth of the number of shoots of chrysanthemum shoot explants with an average age of shoot emergence. 4.17 fruit, whereas in this study by administering 0.1 mg/l into MS media, the shoots gave rise to 3.44 fruit.

Based on table 1 the results of the analysis of variance showed that the interaction treatment with Ferosulfate ($FeSO_4$) and Thiamin gave a significant effect on the number of shoots on Dendrobium sp. The combination of treatments that produced the highest mean value was found in the F2T2 treatment which had more shoots than the single treatment, namely 3.61 fruits, where F2 (Fesoring $FeSO_4$ 27.8 mg/l) functioned in the formation of chlorophyll, the process of forming plant tissue cells and stimulating growth plants. While T2 (Thiamin 0.2 mg/l) functions to increase the activity of hormones contained in the tissue so that it can accelerate the division of new cells.

Table 2. Average shoot height of Dendrobium sp orchid explants treated with ferrous sulfate ($FeSO_4$) and thiamin on MS media (cm)

Factor F				Factor T	Average FT
	Thiamin (0 mg/l)	Thiamin (0.1 mg/l)	Thiamin (0.2 mg/l)	Thiamin (0.3 mg/l)	
Control	1.07 b	0.83 bc	0.78 c	0.87 bc	0.89b
$FeSO_4$ 26.8 mg/l	1.46 ab	0.86 bc	0.68 cd	0.69 cd	0.92b
$FeSO_4$ 27.8 mg/l	0.61 d	0.81 bc	1.48 ab	0.82 bc	0.93b
$FeSO_4$ 28.8 mg/l	1.64 a	1.14 ab	0.99 bc	1.71 a	1.37a
Average T	1.19a	0.91c	0.98bc	1.02b	
KK =8.15 %	BNJ F = 0.09			BNJ T = 0.09	BNJ FT = 0.86

Note: the numbers in the rows and columns followed by the same lowercase letters are not significantly different according to the Significant Difference Advanced Test (BNJ) at the 5% level.

Shoot Height (cm)

Based on the results of observations on the parameter of shoot height of Dendrobium sp orchid explants, after analysis showed that the treatment of ferrous sulfate ($FeSO_4$) and Thiamin alone had a significant effect on shoot height of Dendrobium sp orchid explants, and interacting with ferrosulfate ($FeSO_4$) and Thiamin also significantly affected shoot height of Dendrobium sp orchid plants. The results of the honest significant difference (BNJ) follow-up test at the 5% level can be seen in table 2.

The data in table 2 can be seen that the administration of Fero Sulfate ($FeSO_4$) with the best treatment was found in the administration of $FeSO_4$ 28.8 mg/l MS media, namely with a shoot height of 1.37 cm, further test results with a significant difference (BNJ) at the 5% level showed that the F3 treatment was significantly different from the F2 treatment (30.93 cm), F1 (0.92 cm) and F_0 (0.89 cm). This is because the F3 treatment is the right concentration to be given to Dendrobium sp. Ferosulfate element contained Iron is one of the minerals that are important in the process of forming plant tissue cells. In $FeSO_4$ there are elements of iron and sulfate which play a role in spurring cell division and making chlorophyll in shoots. One way that can be done to increase the Fe content in stimulating the height growth of plant shoots is to increase the concentration of Fe in the planting medium given to the plants (Handayani et al., 2016).

The MS medium used for the nutrient $FeSO_4$ of 28.8 mg/l, giving $FeSO_4$ to the basic media resulted in a shoot height of 2 .5 cm Dendrobium Heterocarpum Lindl orchid plant. Whereas in this study the administration of $FeSO_4$ as much as 27.8 mg/l resulted in a number of leaves of 1.37 cm.

Based on table 2, it shows that giving Thiamin has a significant effect on the parameter of shoot height of Dendrobium sp orchid explants with the best treatment in the treatment of giving Thiamin 0 mg/l into MS media) namely 1.19 cm, from the results of the further difference test honest significant difference (BNJ) at the 5% level indicates that this treatment is different significantly with the treatment of Thiamin 0.1 mg/l to 1.02 cm, the treatment of Thiamin 0.3 mg/l is 0.98 cm and without Thiamin is 0.92 cm.

Giving concentrations without Thiamin gave the best results compared to giving concentrations in other treatments. It is suspected that in MS media there are macronutrients needed in large quantities, one of which is Thiamin. is a vitamin element that is needed by explants for its growth process. Thiamin is useful for stimulating shoot growth, and plays an important role in the formation of shoots in the plant growth phase (Zulkarnain, 2018).

The treatment with Thiamin 0.2 mg/l (T2) produced the least shoot height, this was because when thiamin was used in high concentrations it would inhibit root formation. The inhibition of root formation will disrupt the process of cytokinin synthesis in the roots which will be translocated to the shoots. This will disrupt the formation of new crowns or shoots that will form new leaves. As explained by Siti (2022) cytokinins are believed to be synthesized in roots and translocated via xylem to shoots which aim to form new shoots.

The conclusion that giving 1 mg/l thiamin into MS media had a significant effect on shoot height growth. Chrysanthemum shoot explants with an average shoot height of 4.93 cm, this was due to the different concentrations of the nutrient thiamin given, the resulting response was also different.

Based on table 2 the results of the analysis of variance showed that the interaction treatment with Ferosulfate ($FeSO_4$) and Thiamin gave a significant effect on shoot height in Dendrobium sp. The combination of treatments that produced the highest mean value was in the F3T0 treatment, which was faster in terms of shoot height growth than the single treatment, which was 1.37 cm. The fast growth of shoot height in the F3T0 treatment because both concentrations of the memneric treatment gave a good response to Dendrobium sp. Where F3 (giving $FeSO_4$ 28.8 mg/l) functions in the formation of chlorophyll, the process of forming plant tissue and stimulating plant growth (Salisbury & Cleon, 2019).

Number of Leaves (Streams)

Based on the results of observations on the parameter number of leaves of Dendrobium sp orchid explants, after analysis showed that the treatment of ferosulfate ($FeSO_4$) and Thiamin alone had a significant effect on the number of leaves of Dendrobium sp orchid explants, and by interaction the administration of ferosulfate ($FeSO_4$) and Thiamin also significant effect on the number of explant leaves of Dendrobium sp. The results of the honest significant difference (BNJ) follow-up test at the 5% level can be seen in table 3.

The data in Table 3 can be seen that the administration of Ferro Sulfate ($FeSO_4$) with the best treatment was found in the administration of $FeSO_4$ 26.8 mg/l MS media, namely with a number of shoots of 6.67 strands, further test results significantly different (BNJ) at the 5% level showed that the F1 treatment was significantly different from the F3 treatment (6.14 strands), and F_0 (5.78 strands), but not significantly different from the F2 treatment (6.31 strands).

Treatment F1 with concentrations of $FeSO_4$ (26.8 mg/l) gave the best results compared to the concentrations given to treatments F2, F3 and F_0. This was because the F2 treatment with $FeSO_4$ concentration (26.8 mg/l MS media) was the right concentration to be given to Dendrobium sp.

Giving $FeSO_4$ as much as 27.8 mg/l to MS media produced the highest number of leaves, this is the right concentration to be given to Dendrobium sonia orchid explants. Ferosulfate element contained Iron is one of the minerals that are important in the process of forming plant tissue cells. In $FeSO_4$ there are elements of iron and sulfate which play a role in spurring cell division and making chlorophyll in leaves. One way that can be done for increasing the Fe content in stimulating the growth of plant leaves is through biofortification by increasing the concentration of Fe in Zpt or nutrients given to plants (Panjaitan, 2005).

Treatment Giving $FeSO_4$ 0 mg/l resulted in fewer leaves than F1 because in treatment F0 there was no addition of $FeSO_4$, where $FeSO_4$ is classified as a micro nutrient which plays a role in leaf growth. In $FeSO_4$ there is Iron (Fe) which plays a role in spurring leaf growth. Plant cells and tissues in culture media lack autotrophic ability and therefore, require external $FeSO_4$. The addition of external sources of iron and sulfur to the media increases cell proliferation and optimal leaf regeneration. The concentration of $FeSO_4$ in the media must be sufficient to meet the basic energy requirements for cell division, differentiation and not cause a negative osmotic effect on leaf formation. This shows that the concentration of $FeSO_4$ is one of the factors that control the induction and growth of leaves. Leaf growth is also affected by differences in sucrose concentrations (Gibson & Fuller, 2000).

Table 3. Average number of leaves of Dendrobium sp orchid explants treated with ferrous sulfate ($FeSO_4$) and thiamin on MS media (strands)

Factor F	Factor T				Average FT
	Thiamin (0 mg/l)	Thiamin (0.1 mg/l)	Thiamin (0.2 mg/l)	Thiamin (0.3 mg/l)	
Control	4.78 c	6.11 b	5.67 bc	6.56 ab	5.78c
$FeSO_4$ 26.8 mg/l	7.00 ab	7.00 ab	6.11 b	6.56 ab	6.67a
$FeSO_4$ 26.8 mg/l	5.33 bc	7.22 a	6.22 ab	6.44 ab	6.31ab
$FeSO_4$ 26.8 mg/l	6.33 ab	5.22 bc	6.56 ab	6.44 ab	6.14bc
Average T	5.86b	6.39a	6.14ab	6.50a	
KK = 5.77 %		BNJ F = 0.40	BNJ T = 0.40		BNJ FT = 1.07

Note: the numbers in the rows and columns followed by the same lowercase letters are not significantly different according to the Significant Difference Advanced Test (BNJ) at the 5% level.

The MS media used for the nutrient $FeSO_4$ of 28.8 mg/l, giving $FeSO_4$ to the basic media produces a total of 4 leaves, 41 strands of Dendrobium sp. Whereas in this study the administration of $FeSO_4$ as much as 26.8 mg/l resulted in a total of 6.67 leaves, there was a difference of about 2.26 leaves. This is due to the different concentration of $FeSO_4$ given, the resulting response is also different.

Based on table 3, it shows that giving Thiamin has a significant effect on the parameters of the number of leaves of Dendrobium sp orchid explants. The best treatment is giving Thiamin as much as 0.3 into MS media, namely 5.86 strands, from the results of the further difference test, honest significant difference (BNJ) at level 5 % showed that this treatment was not significantly different from the treatment with Thiamin 0.1 mg/l to ie 6.39 strands, Thiamin 0.2 mg/l ie 6.14 strands, but significantly different from the treatment Without 0 mg/l ie 5.86 strands.

Thiamin concentration (0.3 mg/l) gave the best results compared to T1, T2 and T0 concentrations. This was due to the T2 treatment with Thiamin concentration (0.3 mg/l MS media) being the right concentration to be given to explants of Dendrobium sp. Giving small amounts of Thiamin is able to meet the needs of plants. Even though each plant already has an endogenous ZPT, more vitamins need to be given so that the needs of the plants are properly met (Harjadi, 2019).

Giving thiamin 0 mg/l is the least in the growth of the number of leaves. This is because there is no thiamin administration. Thiamin is one of the elements that plays an important role in accelerating cell division (Garuda, 2015). The rate of cell division that occurs in meristem tissue is influenced by the supply of food materials needed by plants, such as growth regulators and vitamins (Maulia & Basyah, 2021)

The conclusion that the administration of 0.1 mg/l thiamin into MS media had a significant effect on the number of explants of Orchid sandalwood leaves with an average number of leaves 4.93 cm, this is due to the different concentrations of the nutrient thiamin given, the resulting response is also different.

Based on table 3 the results of the analysis of variance showed that the interaction treatment with Ferosulfate ($FeSO_4$) and Thiamin gave a significant effect on the number of leaves on Dendrobium sp. The treatment combination that produced the highest mean value was in the F1T3 treatment, which had more leaves than the single treatment, namely 6.67 leaves. Where F1 (giving $FeSO_4$ 26.8 mg/l) functions in the formation of chlorophyll, the process of forming plant tissue cells and stimulating plant growth. While T3 (Thiamin 0.3 mg/l) functions to increase the activity of hormones found in tissues so that they can accelerate cell division (Garuda, 2015).

The F_0T_0 treatment was the lowest treatment for the number of leaves with an average number of leaves of 0.89, this was due to the absence of $FeSO_4$ and Thiamin in the media. Even though each plant already has endogenous ZPT, more vitamins need to be given so that the plant's needs are properly met (Harjadi, 2019).

Number of roots (Fruits)

Based on the results of observations on the parameters of the number of roots of Dendrobium sp orchid explants, after analysis showed that the treatment of ferrosulfate ($FeSO_4$) alone had a significant effect on the number of roots of Dendrobium orchid explants sp, while the Thiamin treatment alone showed no significant effect and in interaction the administration of ferrosulfate ($FeSO_4$) and Thiamin also did not significantly affect the number of explant roots of Dendrobium sp. The results of the honest significant difference (BNJ) follow-up test at the 5% level can be seen in table 4.

Table 4. Average number of roots of Dendrobium sp orchid explants treated with ferrous sulfate ($FeSO_4$) and thiamin on MS media (fruit)

Factor F	Factor T				Average FT
	Thiamin (0 mg/l)	Thiamin (0.1 mg/l)	Thiamin (0.2 mg/l)	Thiamin (0.3 mg/l)	
Control	4.11	4.33	4.56	5.22	4.56b
$FeSO_4$ 26.8 mg/l	3.56	4.67	4.22	3.89	4.08c
$FeSO_4$ 26.8 mg/l	4.56	5.11	5.78	4.33	4.94b
$FeSO_4$ 26.8 mg/l	6.67	4.11	4.89	5.00	5.17a
Average F	4.72	4.56	4.86	4.61	
KK = 6.33 %			BNJ F = 0.30		

Note: the numbers in the rows and columns followed by the same lowercase letters are not significantly different from the follow-up test of significant differences (BNJ) at the 5% level.

From the data in table 4 it can be seen that the administration of KH_2PO_4 with the best treatment was in the administration of $FeSO_4$ 28.8 mg/l MS media, namely with a number of roots of 5.17 fruit, further test results significantly different (BNJ) at the 5% level showed that the F3 treatment was different significantly with F2 (4.94 pieces) and F_0 (4.56 pieces), and F1 (4.08 pieces).

Concentration of $FeSO_4$ (28.8 mg/l to MS medium) gave the best results compared to other concentrations of

treatment, this was because treatment with $FeSO_4$ concentration (28.8 mg/l to MS medium) was the right concentration to be given to explants. Orchid Dendrobium sp. Elements of iron and sulfur contained in $FeSO_4$. The function of iron (Fe) plays a role in the formation of chlorophyll (Zastepa et al., 2021). Therefore the optimal availability of Fe is needed by plants. If there is not enough Fe in the nutrient solution, then the formation of chlorophyll will not be perfect, respiration is not optimal and the energy produced is only a little so that the absorption of nutrients by roots is slow. As a result, plant growth stagnates or stops**.** Sulfur in orchids is needed for the synthesis of the amino acids cystine, cysteine, and methionine, which in turn form proteins. In addition, sulfur is very helpful for the development of shoots, roots and tillers.

MS media which was used for the nutrient element $FeSO_4$ as much as 28.8 mg/l, the application of $FeSO_4$ to the basic media resulted in a total number of roots of 1.02 Dendrobium sp. Whereas in this study the administration of 28.8 mg/l $FeSO_4$ resulted in a total of 5.15 leaves. This is due to the different concentration of $FeSO_4$ given, the resulting response is also different.

Based on table 4, it shows that the administration of Thiamin was not significantly different on the parameters of the number of roots of Dendrobium sp. orchid explants. This is presumably because the concentration of the nutrient Thiamin given has not been able to give a good response to the number of roots of Dendrobium sp. orchid explants. However, if seen from the average value, which produces more leaves in this study, it was obtained by giving a concentration of Thiamin (0.2 mg/l), namely 4.86 fruit, followed by no thiamin, namely 4.72 fruit, Thiamin 0.3 mg/ l which is 4.61 pieces Thiamin 0.1 mg/l which is 4.56 pieces. The provision of Thiamin nutrients has not been able to meet the needs of plants. Even though each plant already has endogenous ZPT, more nutrients need to be given so that the plant's need for the right nutrients can be fulfilled properly.

Based on table 4 the results of the analysis of variance showed that the interaction treatment of Ferosulfate ($FeSO_4$) and Thiamin administration was significantly different on the number of roots in Dendrobium sp. Judging from the average treatment that produced the highest mean value in the F3T2 treatment, there were more roots than the single treatment, namely 5.17 pieces. Where F3 ($FeSO_4$ 28.8 mg/l) functions in the formation of chlorophyll, the process of forming plant tissue cells and stimulating plant growth.

Root length (cm)

Based on the results of observations on the parameters of the root length of Dendrobium sp orchid explants, after analysis showed that the treatment of ferosulfate ($FeSO_4$) and Thiamin alone had a significant effect on the root length of Dendrobium sp orchid explants, and by interaction the administration of ferosulfate ($FeSO_4$) and Thiamin also significant effect on the root length of Dendrobium sp. orchid plant explants. The results of the honest significant difference test (BNJ) at the 5% level can be seen in table 5.

Table 5. Average root length of Dendrobium sp orchid explants treated with Ferosulfate ($FeSO_4$) and Thiamin on MS media (cm)

Factor F				Factor T	Average F
	Thiamin (0 mg/l)	Thiamin (0.1 mg/l)	Thiamin (0.2 mg/l)	Thiamin (0.3 mg/l)	
Control	1.43 b	1.09 bc	1.37 bc	1.19 bc	1.27a
$FeSO_4$ 26.8 mg/l	1.41 bc	1.12 bc	0.71 d	1.30 bc	1.14b
$FeSO_4$ 26.8 mg/l	1.08 c	1.24 bc	1.78 a	0.88 cd	1.24ab
$FeSO_4$ 26.8 mg/l	1.56 a	1.21 bc	1.24 bc	1.16 bc	1.29a
Average T	1.37a	1.17bc	1.28ab	1.13c	
KK = 9.23 %		BNJ F =0.13	BNJ T= 0.13		BNJ FT = 0.34

Note: the numbers in the rows and columns followed by the same lowercase letters are not significantly different according to the Significant Difference Advanced Test (BNJ) at the 5% level.

The data in table 5 can be seen that the administration of Ferro Sulfate ($FeSO_4$) with the best treatment was found in the administration of $FeSO_4$ 28.8 mg/l MS media, namely with a root length of 1.29 cm, the results of the further test were significantly different (BNJ) at the 5% level showed that the F2 treatment was significantly different from the F1 treatment (1.14 cm) and F2 (1.24 cm), but not significantly different from the F_0 treatment (1.27 cm).

The treatment of $FeSO_4$ as much as 28.8 mg/l to MS media produced the best root length, this was the right concentration to be given to Dendrobium sp. Ferosulfate element contained Iron is one of the minerals that are important in the process of forming plant tissue cells. One way that can be done to increase the Fe content in stimulating plant growth is through biofortification by increasing the concentration of Fe in Zpt or nutrients given to plants (Handayani et al., 2016).

Giving $FeSO_4$ 26.8 mg/l produced the least root length, namely 1.14 cm, because in the F1 treatment a little added $FeSO_4$ nutrients, it was necessary to give the right concentration in order to accelerate root growth,

because $FeSO_4$ is a micro element that plays an important role in explant growth. In $FeSO_4$ there are elements of iron and sulfate which play a role in spurring cell division and making chlorophyll in leaves. If there is a deficiency of $FeSO_4$, it will inhibit root growth.

If this study is compared with a study conducted by Gunawan et al (2021) then the results are different, in the MS medium used for the nutrient $FeSO_4$ of 28.8 mg/l, giving $FeSO_4$ to the basic media produces a root length of 1 .26 cm Dendrobium bifalce orchid plant. Whereas in this study the administration of $FeSO_4$ as much as 28.8 mg/l resulted in a root length of 1.29 cm. This is due to the different concentration of $FeSO_4$ given, the resulting response is also different.

Based on table 5, it shows that giving Thiamin has a significant effect on the root length parameters of Dendrobium sp orchid explants with the best treatment in the treatment of giving Thiamin 0 mg/l into MS media which is 1.37 cm, from the results of the further difference test honest significant difference (BNJ) on The 5% level indicated that the T0 treatment was significantly different from the treatment with Thiamin 0.2 mg/l to 1.28 cm, but not significantly different from the treatment with Thiamin 0.1 mg/l, namely 1.17 cm and Thiamin 0. 2 mg/l is 1.13 cm.

Thiamin concentration (0 mg/l) gave good results compared to the concentration in T2, T1 and T3 treatments. Vitamins have an important role for the growth of every plant. Vitamins are also needed by plants, because vitamins can be used in a short time for vegetative growth, especially the development of roots, shoots and leaves.

The treatment with Thiamin 0.3 resulted in the least root length, this was because when thiamin was used in high concentrations it would inhibit root formation. The inhibition of root formation will disrupt the process of cytokinin synthesis in the roots which will be translocated to the shoots. This will disrupt the formation of new crowns or shoots that will form new leaves. As explained by Siti (2022) cytokinins are believed to be synthesized in roots and translocated via xylem to shoots which aim to form new roots.

The conclusion that the administration of 2 mg/l thiamin into MS media had a significant effect on the height growth of chrysanthemum shoot explants with an average shoot height of 2 .34 cm, while in the research giving without Thiamin was able to produce a root length of 1.37 cm. this is due to the different concentrations of the nutrient thiamin given, the resulting response is also different.

Based on table 5 the results of the analysis of variance showed that the interaction treatment with Ferosulfate ($FeSO_4$) and Thiamin gave a significantly different effect on root length in Dendrobium sp. The combination of treatments that produced the highest mean value was in the F3T0 treatment, which had longer roots than the single treatment, namely 1.37 cm, where F3 (Fesorization of $FeSO_4$ 28.8 mg/l) functioned in the formation of chlorophyll, the process of forming plant tissue cells and stimulating plant growth (Yusnita, 2017).

Conclusion

By giving various concentrations of ferrosulfate ($FeSO_4$), 27.8 mg/l ms media individually was significantly different on the parameters of shoot height, number of roots, and root length, where the best treatment was in F3 with an average shoot height of 6.14 cm, number of roots 1 .29 pods, and root length 3.03 cm, F2 treatment for the parameter number of shoots with an average number of shoots 3.61 pods and the F1 treatment was significantly different for the parameter number of leaves with an average number of 6.67 strands in Dendrobium orchid explants sp. By giving various concentrations of Thiamin individually affects the shot quantity parameter with the best treatment found in T2 with an average (3.44 pieces), T0 for the parameter of shoot height (0.98 cm), T3 for the parameter of the number of leaves (6.14 pieces), T0 for the root length parameter (1.28 cm). The interaction treatment with ferrous sulfate ($FeSO_4$) and Thiamin concentrations interacted significantly with the experimental parameters, including the number of shoots meter with F3T3 treatment ($FeSO_4$ 28.8 mg/l and Thiamin 0.3 mg/l) with an average number of shoots of 1.71. In interaction, the administration of Ferosulfate ($FeSO_4$) and Thiamin had a significant effect on the parameters observed, namely the number of shoots treated with F3T3 ($FeSO_4$ 28.8 mg/l and Thiamin 0.3 mg/l) with an average total shoot 1.71 (ea).

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