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The Effect of Microalgae Extract as a Biostimulant on Arabica Coffee (*Coffea arabica* L.) Callus Induction

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Abstract: Arabica coffee (*Coffea arabica* L.) is an economically important plantation crop in Indonesia, but its productivity is still relatively low. The purpose of this study was to influence *Chlorella vulgaris* extract as a biostimulant on *in vitro* callus induction using Arabica coffee leaf disc explants. The study method used a completely randomized design with six Murashige and Skoog (MS) media formulations consisting of synthetic plant growth regulator (PGR) (2,4-D and kinetin), 15% and 20% *Chlorella vulgaris* extract, and a combination of microalgae extract with PGR. The parameters observed included the percentage of explants with callus, color and texture, and explant contamination. The results and discussion of the media formulation significantly affected callus induction, while contamination was not significantly affected. PGR media and 15% *Chlorella vulgaris* extract treatment produced comparable callus formation percentages, indicating that microalgae extracts were as effective as synthetic PGRs in supporting early callus induction. Most of the calli were yellow to greenish in color with a compact texture, indicating the suitability of the callus induction stage, although further optimization is needed to obtain easily disintegrated calli for subsequent somatic embryogenesis. The conclusion shows the potential of *Chlorella vulgaris* extract as an environmentally friendly biostimulant for Arabica coffee tissue culture.

Keywords: Callus induction; *Coffea arabica;* Microalgae biostimulant; Microalgae extract; Tissue culture.

Introduction

Arabica coffee (*Coffea arabica* L.) is Indonesia's primary plantation commodity. According to Statistics Indonesia (BPS), Indonesia's average coffee productivity was approximately 0.82 ton ha^{-1} in 2023. Although Indonesia is the world's fourth-largest coffee producer, its productivity remains lower than that achieved in intensive coffee production systems in Brazil and Vietnam. Indonesia's low coffee productivity is partly due to the low quality of coffee planting material, characterized by low viability and uneven plant growth (Torok et al., 2018). Increasing national coffee productivity can be achieved through mass production of coffee seedlings *in vitro.*

Coffee plantlets can be mass propagated through somatic embryogenesis, in which callus induction represents an important initial stage for the development of embryogenic tissues. Callus is a mass of undifferentiated plant cells consisting of unorganized, thin-walled, actively dividing parenchymal cells. This is exemplified by Di Bonaventura et al. (2024) who successfully induced friable callus in coffee by administering 2,4-D and kinetin at a concentration of 1 ppm.

To date, the use of plant growth regulator (PGR) has been a common standard procedure for callus induction, including in coffee. The use of synthetic PGR offers advantages such as consistent results, practicality, and compatibility with a wide range of explants (Etienne et al., 2009). However, the use of synthetic PGR also

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carries risks, including phytotoxicity, somaclonal variation, negative environmental residues, and high production costs (Bairu et al., 2011). In this context, the use of alternatives to synthetic PGR needs to be explored, one example being microalgae extract-based biostimulants.

Microalgae extract-based biostimulants have the potential to be used as supplements or substitutes for synthetic plant growth regulator (PGR). Biostimulants are biological substances that can stimulate certain physiological processes in plants (Jardin, 2015). In the context of tissue culture, biostimulants can enhance morphogenesis responses through organogenesis or somatic embryogenesis, reduce *in vitro* stress, and improve plantlet vigor and quality (Yakhin et al., 2017). Microalgae are a group of microbes that live in various aquatic ecosystems and are characterized by the ability to photosynthesize, possess chlorophyll, and synthesize various beneficial compounds (Borowitzka, 2013).

The biochemical composition of microalgae is strongly influenced by nutrient availability during cultivation. Variations in nitrogen sources may alter the accumulation of proteins, pigments, and other bioactive metabolites that contribute to their potential as plant biostimulants (Zsalzsabil, 2023). Green microalgae such as *Chlorella* and *Scenedesmus* are known to contain various bioactive compounds, including amino acids, vitamins, carbohydrates, polysaccharides, fatty acids, photosynthetic pigments, and phytohormones such as auxins, cytokinins, gibberellins, and abscisic acid. In addition, *cyanobacteria* commercially known as *Spirulina* (*Arthrospira* sp.) have also been reported to exhibit biostimulant properties despite belonging to a different taxonomic group. The composition and concentration of these bioactive compounds vary considerably among species, depending on taxonomy, cultivation conditions, and extraction methods. These compounds are believed to modulate plant metabolism and physiological signaling in *in vitro* cultures (Ronga et al., 2019).

Several studies have examined the use and effectiveness of microalgae extracts as biostimulants in in vitro plant cultures. Corbellini et al. (2020) found that *Chlorophyceace* sp. microalgae extract was effective in inducing and regenerating *protocorm-like bodies* (PLB) in *Cattleya labiata* orchids. Meanwhile, Ronga et al. (2019) identified the physiological and morphogenetic responses of *Solanum lycopersicum* plants through supplementation with *Chlorella vulgaris* microalgae extract in MS medium. These findings indicate the initial potential use of microalgae extracts as biostimulants in plant tissue culture. Although previous studies have demonstrated the potential of microalgae extracts as biostimulants in several plant species, information regarding their application during *in vitro* callus induction of *Coffea arabica* remains very limited.

Coffee tissues harbor diverse endogenous microorganisms and metabolites that influence physiological characteristics and tissue responses during in vitro culture. Characterization of coffee-associated microorganisms may therefore provide additional insight into biological factors influencing tissue culture performance (Hasanuddin et al., 2023). In particular, the effectiveness of *Chlorella vulgaris* extract as a supplement or partial substitute for synthetic plant growth regulators during the early stage of coffee callogenesis has not been comprehensively investigated. Therefore, this study provides new evidence regarding the use of *Chlorella vulgaris* extract as a sustainable biostimulant for Arabica coffee tissue culture, supporting the development of more environmentally friendly propagation technologies while reducing dependence on synthetic plant growth regulators. However, similar studies are still very limited, especially regarding their application as biostimulants in in vitro Arabica coffee cultures. Therefore, this study aims to examine the effect of microalgae extract biostimulant supplementation on Arabica coffee callus induction.

Method

This research was conducted in August-November 2025 at the Biotechnology Laboratory, Faculty of Agriculture, National Development University Veteran East Java. The incubation room had microclimate conditions: temperature 22-27^{o}C, humidity 60%, and light intensity of 2,500 lux from white LED lamps. The microalgae used were *Chlorella vulgaris* sp. obtained from cultur. The explants used were taken from young leaves of Arabica coffee variety Kartika 1. Other materials used were MS media, PGR 2,4-D (auxin) and kinetin (cytokinin), sterilants (Tween-20, fungicide, 70% alcohol, and sterile distilled water), as well as other standard tissue culture materials and equipment.

The experiment was designed as a completely randomized design with one research factor, namely the formulation of callus induction media consisting of six formulations, namely: MS + 2,4-D 1ppm + kinetin 1ppm (PGR); microalgae extract 15% (MA15%); microalgae extract 20% (MA20%); microalgae extract 10% + PGR; microalgae extract 15% + PGR; microalgae extract 20% + PGR. Each treatment was repeated three times where each treatment unit consisted of three culture bottles containing two explants per bottle.

The preparation of microalgae extract biostimulants was carried out by homogenizing the *Chlorella vulgaris* sp. microalgae, then centrifuging at 3000–5000 rpm for 10–15 minutes to separate the cells

from the medium. After the cell sediment formed at the bottom, the upper liquid (supernatant) was carefully removed using a pipette and was ready for use. The callus induction medium was then prepared based on the formulation applied by following the standard procedure for making plant tissue culture media. Explant preparation was carried out by taking Arabica coffee leaf samples, then sterilizing the explants first by washing them with running water and soaking them in liquid soap for 1 minute. Next, the explants were soaked in a Tween-20 solution for 10 minutes, a Dithane-45 solution for 15 minutes, and 70% alcohol for 1 minute. Each stage was followed by three washings using sterile distilled water until the explants were ready for use for tissue culture aseptically. Once ready, the explants were then initiated in culture bottles and incubated for 90 days. Maintenance includes cleaning the incubation racks of dust and spraying them with 70% alcohol periodically, as well as fumigating the incubation room every two weeks. Furthermore, if dead or contaminated cultures are found, they are immediately removed from the incubation room to prevent the spread of infection to other culture bottles.

The experimental parameter data collected included the percentage of callus explants (%), the color and texture of callus explants, and the percentage of contaminated explants (%). Numerical data were analyzed using ANOVA followed by BNJ test, if the assumptions of normality and homoscedasticity were met. The Welch ANOVA test accompanied by the non-parametric Games-Howell test was performed if the assumptions of homoscedasticity were not met. Meanwhile, if both assumptions of normality and homoscedasticity were not met, the analysis was performed non-parametrically using the Kruskall-Wallis test and the Dunn's advanced test. Statistical analysis was performed using R-Studio version 3.6.0 with a significance level of 5% (*p-value* = 0.05). Descriptive analysis was performed on non-numerical data, namely the color and texture of callus.

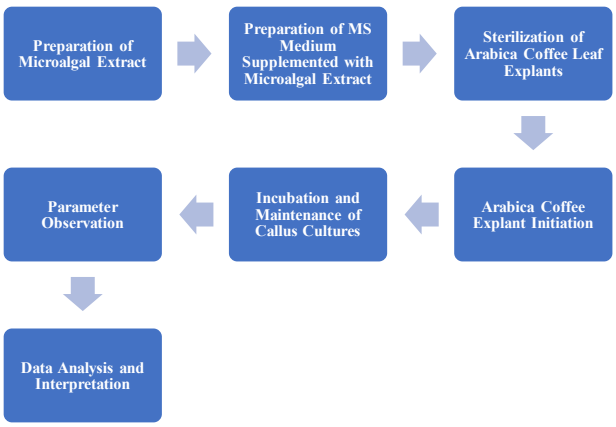

Figure 1. Research Methodology

Result and Discussion

Percentage of Explant Growth (%)

Parameters of the percentage of explants with callus, ANOVA showed significant differences between media formulations. The highest percentage was obtained in the formulation of PGR 2,4-D + Kinetin which was not significantly different from the MA15%, MA20% and PGR + MA15% extracts. The Effect of Microalgae Extract as a Biostimulant on Arabica Coffee (*Coffea arabica* L.) Callus Induction.

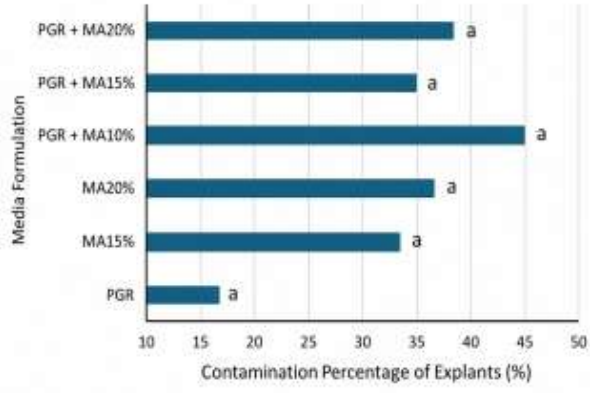

Figure 2. Percentage of callus explants (%) in various culture media formulations

Similar findings were reported by Arifiana et al. (2026), who demonstrated that modification of MS medium composition significantly affected callus induction percentage, callus morphology, and fresh weight of Arabica coffee callus. The results of the study showed that the media formulations had different effects on the percentage of explants forming callus. These findings indicate that the composition of the culture medium plays a decisive role during the early stage of callus induction by regulating cellular dedifferentiation and subsequent cell proliferation (Fehér, 2019; Ikeuchi et al., 2016). Based on the graph, the PGR and 15% MA treatment resulted in the highest percentage of explants forming callus, while the PGR + 10% MA treatment showed the lowest. The finding that the 15% MA treatment produced a callus induction percentage comparable to the PGR treatment suggests that *Chlorella vulgaris* extract may serve as a potential natural biostimulant during the initial stage of callogenesis. Similar observations have been reported in previous studies, where natural biostimulants containing phytohormones and bioactive metabolites promoted cell division and tissue differentiation during *in vitro* culture (Colla & Rouphael, 2020; Ronga et al., 2019).

However, the effectiveness of these compounds is highly dependent on their concentration and interaction with endogenous plant hormones (Fehér, 2019). The

high percentage of callus formation in media containing plant growth regulator (PGR) indicates that the presence of plant hormones, particularly auxin and cytokinin, plays a crucial role in stimulating cell dedifferentiation and cell division, resulting in the formation of callus tissue. This process occurs because PGRs are able to induce physiological changes in plant cells, causing them to return to meristematic behavior and actively divide (Ikeuchi et al., 2016). The comparable response observed in the 15% MA treatment suggests that the endogenous bioactive compounds present in *Chlorella vulgaris* extract may partially substitute the function of synthetic plant growth regulators during the early stage of callus induction. This finding indicates that microalgae biostimulants have the potential to support cellular reprogramming and cell proliferation required for callogenesis, although their effectiveness depends on the concentration applied (Colla & Rouphael, 2020; Kapoore et al., 2021).

Microalgae are known to contain various bioactive compounds, including phytohormones, amino acids, vitamins, polysaccharides, and antioxidants, which function as natural biostimulants to promote plant growth and morphogenesis (Chiaiese et al., 2018; Colla & Rouphael, 2020). In addition, these compounds have been reported to enhance nutrient uptake, improve cellular metabolism, and stimulate endogenous hormonal signaling during *in vitro* culture (González-Pérez et al., 2022; Jardin, 2015). Besides improving plant metabolism, microalgae extracts have also been reported to enhance stress tolerance and antioxidant activity, thereby maintaining cellular viability during *in vitro* culture. These physiological responses are beneficial during the initial stages of callus induction, when explants experience considerable stress due to wounding and adaptation to artificial culture conditions (Parmar et al., 2023; Ronga et al., 2019).

The results of this study indicate that the combination of plant growth regulator (PGR) with specific microalgae concentrations does not always increase callus formation. In the PGR + 10% MA and PGR + 20% MA treatments, the percentage of explants with callus tended to be lower than in the PGR or 15% MA treatments alone. One possible explanation is that excessively high concentrations of bioactive compounds may disrupt hormonal homeostasis in the culture medium, thereby reducing the balance between endogenous and exogenous growth regulators required for efficient callus induction (Fehér, 2019; Ikeuchi et al., 2016). A similar phenomenon has been reported in several plant tissue culture studies, where excessive concentrations of exogenous growth-promoting compounds disturb hormonal homeostasis, resulting in reduced callus induction efficiency and abnormal tissue development (Fehér, 2019; Ikeuchi et al., 2016). Hormonal imbalances can inhibit cell dedifferentiation and disrupt cell division, which is necessary for callus formation (Ikeuchi et al., 2016). In addition, the relatively low percentage of callus formation observed in the PGR + 10% MA treatment may also be associated with its higher contamination level. Contamination can reduce explant viability and inhibit cell proliferation, thereby limiting callus initiation (Leelavathy & Deepa Sankar, 2016). Therefore, the lower percentage of callus formation in this treatment was likely influenced not only by the culture medium formulation but also by contamination during culture.

From an applied perspective, the ability of 15% *Chlorella vulgaris* extract to induce callus at a level comparable to synthetic plant growth regulators offers promising opportunities for developing more sustainable tissue culture protocols. Natural biostimulants derived from microalgae are renewable, biodegradable, and environmentally friendly, making them attractive alternatives for reducing the reliance on synthetic chemicals in plant biotechnology. Their application may contribute to more sustainable propagation systems for economically important crops such as coffee while supporting environmentally responsible agricultural practices (Colla & Rouphael, 2020; González-Pérez et al., 2022).

Previous research also reported that microalgae extracts can enhance plant growth and development due to their content of natural growth regulators such as auxins, cytokinins, and gibberellins. These compounds can stimulate tissue growth, increase cell metabolism, and support the process of new tissue formation in plants. Therefore, the use of microalgae extracts in culture media can act as biostimulants that support callus formation and plant tissue growth *in vitro* (Ronga et al., 2019). Overall, the present findings demonstrate that supplementation with 15% Chlorella vulgaris extract was capable of producing callus induction comparable to that obtained using synthetic plant growth regulators. This result highlights the potential application of microalgae-based biostimulants as a more sustainable alternative for coffee tissue culture by reducing dependence on synthetic growth regulators while maintaining satisfactory callogenesis. However, further studies involving endogenous hormone analysis and embryogenic callus development are required to clarify the physiological mechanisms underlying this response.

Callus Color and Texture

The callus morphological characteristics observed included color based on the Munsell Color Chart standard and callus texture in various media

formulations. The results showed that the resulting color varied from grayish yellow, lime green, greenish yellow, to reddish brown. Most callus had a compact texture, while in the 20% MA treatment, it had a friable texture.

Table 1. Morphological characteristics of callus in various culture media formulations

Treatment	Color	Munsell Collor	Picture	Texture
PGR (Control)		2.5 GY 5/6 Grayish Yellow		Compact
MA 15%		5GY 6/8 Lime Green		Compact
MA 20%		10R 2/6 Dark Reddish Brown		Friable
PGR + MA 10%		2.5GY 7/10 Greenish Yellow		Compact
PGR + MA 15%		2.5GY 5/6 Greenish Yellow		Compact
PGR + MA 20%		5YR 5/8 Reddish Brown		Compact

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Observations show that media formulations vary the morphological characteristics of the resulting callus, both in terms of color and texture. In this study, the resulting callus color varied from grayish yellow, lime green, greenish yellow, to reddish brown. Variations in callus color in plant tissue culture are generally related to cellular physiological activity, chlorophyll accumulation, phenolic compound oxidation, and the metabolic status of cultured tissues (Fehér, 2019; Ikeuchi et al., 2016). Brightly colored callus, such as yellow or green, usually indicates actively dividing cells with good viability (George & Hall, 2008), while brown callus tends to indicate the presence of phenolic oxidation processes that can inhibit tissue growth (Ikeuchi et al., 2016). Similar observations were reported by Ren et al. (2020), who demonstrated that suppression of browning was associated with improved callus quality and cellular viability. Callus color is widely recognized as an important morphological indicator of tissue quality during *in vitro* culture. Yellow to green callus generally reflects active cell division, balanced metabolic activity, and a relatively healthy physiological condition, whereas brown callus is frequently associated with oxidative stress and the accumulation of oxidized phenolic compounds released from wounded explants (Chen et al., 2019).

In the PGR treatment (control), the callus formed was grayish-yellow in color with a compact texture. A compact callus texture indicates that the cells composing the callus are tightly arranged and have relatively stable cell division activity. A compact texture is often found in callus cultures that are in media conditions that meet the needs of plant tissue growth (Fehér, 2019). Furthermore, compact callus is generally considered an indicator of good tissue organization and has been associated with a greater potential for subsequent organogenesis or somatic embryogenesis, depending on the plant species and culture conditions (Fehér, 2019; Ikeuchi et al., 2016). In addition, the greenish coloration observed in several treatments, such as MA15% and PGR + MA10%, suggests chlorophyll biosynthesis and active plastid differentiation, reflecting favorable metabolic activity during *in vitro* culture (George & Hall, 2008; Ikeuchi et al., 2016).

The 20% MA treatment produced callus with a dark reddish brown color and a friable texture. Nevertheless, friable callus does not always indicate poor tissue quality. In several species, friable callus has been reported to possess higher cell proliferation rates and is frequently preferred for cell suspension culture and somatic embryogenesis, provided that cell viability is maintained (Fehér, 2019; George & Hall, 2008). The browning of callus is usually caused by the oxidation of phenolic compounds released by plant tissue during the culture process. The browning process often occurs in woody plant tissue cultures and can inhibit callus growth if excessive (Permadi et al., 2023). This phenomenon is particularly common in woody species such as *Coffea arabica*, where wounded explants release phenolic compounds that are subsequently oxidized by polyphenol oxidase and peroxidase enzymes. Excessive phenolic oxidation may reduce cell viability, inhibit cell division, and ultimately suppress callus development (Chen et al., 2019). Furthermore, the friable texture of callus indicates that callus cells are looser and less tightly packed, which is often associated with faster callus growth but a less compact tissue structure.

Although the callus exhibited a dark reddish-brown color, which is commonly associated with phenolic oxidation, the tissue remained friable during the observation period. This suggests that browning had occurred without completely eliminating cell viability. However, further viability or histological analyses are required to verify whether the callus retained embryogenic potential. In the combination treatment of plant growth regulator (PGR) and microalgae extract, most calli exhibited a compact texture with a greenish-yellow to reddish-brown color. This indicates that the addition of microalgae extract to the culture medium may influence plant cell metabolic activity. Microalgae are known to contain various bioactive compounds such as amino acids, vitamins, and natural growth regulators

that can act as biostimulants for plant growth. These compounds can increase cell physiological activity and support cell division in plant tissue culture (Chiaiese et al., 2018; Colla & Rouphael, 2020; Ronga et al., 2019).

The predominance of compact callus with greenish-yellow coloration in most treatments indicates that the culture media provided favorable physiological conditions for callus development. This observation suggests that microalgae supplementation at appropriate concentrations may support cellular metabolism and maintain tissue viability without inducing severe oxidative stress, thereby contributing to successful callus formation (Chiaiese et al., 2018; Colla & Rouphael, 2020).

In general, callus morphological characteristics such as color and texture can be used as indicators of tissue culture success. Brightly colored callus with a compact texture generally indicates good physiological condition and has a higher potential to progress to the plant regeneration stage. Therefore, the observed differences in callus morphology among treatments not only reflect the response of explants to different media formulations but also indicate differences in cellular physiological status throughout the culture period. The friable callus observed in the 20% MA treatment may indicate a greater potential for cell proliferation and subsequent somatic embryogenesis, although further histological or regeneration studies are required to confirm its embryogenic nature (Fehér, 2019). These findings demonstrate that morphological evaluation provides an effective preliminary approach for assessing callus quality prior to histological examination or regeneration assays. The predominance of compact and light-colored callus in the PGR and 15% MA treatments is consistent with their higher percentages of callus induction, suggesting that these media formulations not only promoted callus initiation but also supported the development of morphologically healthy callus.

Percentage of Contaminated Explants (%)

Regarding the percentage of contaminated explants, ANOVA showed that media formulation had no significant effect on the level of explant contamination. The highest percentage of contamination was obtained with the PGR + 10% MA formulation, while the lowest percentage was found with the PGR treatment. However, statistically, all treatments did not differ significantly from each other in affecting the level of explant contamination.

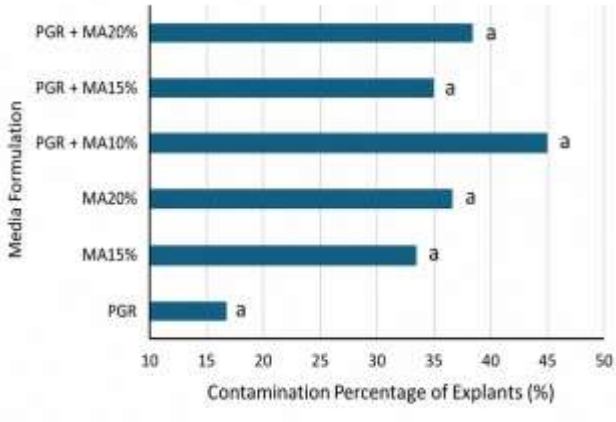

Figure 3. Percentage of contaminated explants (%) in various culture media formulations

Based on observations, the percentage of contaminated explants in various media formulations ranged from approximately 17–45%. The BNJ test results showed that all treatments had the same letter, thus it can be concluded that the media formulation did not significantly affect the level of explant contamination. This indicates that contamination in plant tissue culture is more influenced by technical factors during the culture process, such as the explant sterilization method, equipment cleanliness, and work environment conditions during the inoculation process.

Contamination is a major problem in plant tissue culture techniques because nutrient-rich culture media strongly supports the growth of microorganisms such as bacteria and fungi. Similar findings have been reported by Permadi et al. (2023), who stated that microbial contamination and tissue browning remain the two major limiting factors affecting the success of plant tissue culture. These microorganisms can grow faster than plant tissue, inhibiting growth and even causing explant death. Therefore, the success of tissue culture depends heavily on aseptic conditions and the effectiveness of the sterilization process during the explant preparation and planting stages (Leelavathy & Deepa Sankar, 2016).

Besides originating from the explant surface, contamination can also originate from endophytic microorganisms found within plant tissue. These microorganisms often cannot be completely eliminated through surface sterilization, allowing them to emerge and grow during the culture process. Furthermore, other sources of contamination can come from the culture medium, the equipment used, the air in the culture room, or technical errors during the inoculation process (Liu et al., 2024).

In this study, treatments containing a combination of PGR and microalgae tended to show a higher percentage of contamination than treatments containing

PGR alone. This is likely because the culture medium containing additional organic compounds can provide a nutrient source for microorganisms, thus supporting the growth of contaminants. Tissue culture media generally contain sugars and other nutrients that can also be utilized by microorganisms. Therefore, if the sterilization process is less than optimal, microorganisms will grow rapidly in the culture medium (Balo, 2023).

Thus, controlling contamination in tissue culture relies heavily on explant sterilization techniques, the use of sterile equipment, and the application of proper aseptic techniques in laminar air flow. Proper aseptic procedures will help reduce contamination levels, thereby increasing tissue culture success.

Testing the Assumptions of Normality and Homoscedasticity

Results of the Normality and Homoscedasticity Assumption Test based on the Shapiro Wilk test (H_0: data are normally distributed, H_1: data are not normally distributed, *p-value* > 0.05); homoscedasticity test based on the Levene test (H_0: variances are homogeneous, H_1: variances are not homogeneous, *p-value* > 0.05); * indicates that H_0 is significant at the 5% level for all numerical data.

Table 2. Normality test based on Shapiro Wilk

Parameter	Test Statistic and *p-value*		Interpretation and Follow-up
	Normality Test	Homoscedasticity Test	
Percentage of Callus-Forming Explants	W = 0.98869 *p-value* = 0.9828	F = 1.3671 *p-value* = 0.2715	Since both assumptions are met, an ANOVA test is performed.
Percentage of Contaminated Explants	W = 0.97646 *p-value* = 0.7257	F = 1.9688 *p-value* = 0.12	Since both assumptions are met, an ANOVA test is performed.

Before conducting the analysis of variance (ANOVA), basic statistical assumptions were first tested, namely the normality test and the homoscedasticity test. This test aims to ensure that the data obtained meets the requirements for using parametric analysis so that the results can be interpreted validly. The normality test in this study used the Shapiro–Wilk method, while the homoscedasticity test used the Levene test.

Based on the analysis results on the percentage of explants with callus, the Shapiro–Wilk test statistic value was obtained as W = 0.98869 with a *p-value* = 0.9828. A *p-value* greater than 0.05 indicates that the data is normally distributed. In addition, the results of the homoscedasticity test showed an F value of 1.3671 with a *p-value* = 0.2715, which is also greater than 0.05. This indicates that the data variance between treatments is homogeneous. By fulfilling these two assumptions, the data on this parameter meets the requirements for further analysis using analysis of variance (ANOVA).

In the parameter of the percentage of contaminated explants, the results of the normality test showed a value of W = 0.97646 with a p-value = 0.7257, which indicates that the data is normally distributed because the *p-value* is greater than 0.05. Furthermore, the results of the homogeneity of variance test showed a value of F = 1.9688 with a p-value = 0.12, which is also greater than 0.05. This indicates that the variance between treatments is homogeneous. Thus, both assumptions of parametric analysis on this parameter have also been met so that analysis of variance (ANOVA) can be carried out to determine the effect of treatment on the observed parameters.

Testing the assumptions of normality and homogeneity is a crucial step in statistical analysis in experimental research. Data that meets the assumptions of normality and homoscedasticity allow for the use of parametric analyses such as ANOVA, which have greater statistical power in detecting differences between treatments. If both assumptions are met, the analysis of variance results are considered valid and can be used as a basis for drawing research conclusions.

Conclusion

Based on the research results, culture media formulation significantly affected callus induction in Coffea arabica leaf explants. The 15% *Chlorella vulgaris* extract treatment produced a callus induction response comparable to the synthetic PGR treatment, with no significant difference between the two treatments, indicating its potential as a partial substitute for synthetic plant growth regulators. This indicates that the microalgae extract of *Chlorella vulgaris* has the potential to be used as a biostimulant to support callus formation in Arabica coffee tissue culture. The callus formed was generally yellow to greenish in color with a predominantly compact texture, indicating stable cell division during the initial stage of callus induction. Meanwhile, the media formulation did not significantly affect the level of explant contamination, so contamination was more influenced by technical factors

such as the sterilization process and aseptic working conditions.

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Author Contributions
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Conflicts of Interest
The authors declare that there are no conflicts of interest related to this research. This research was conducted independently and was not influenced by personal interests, professional relationships, or financial affiliations. All decisions made during the research and writing process were made objectively and with scientific integrity.

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