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Improving The Growth And Adaptation Of The Black Orchid Plantlet (*Coelogyne Pandurata* Lindl) In Various Growing Media by Giving Plant Extracts as Biostimulants at The Acclimatization Stage

Zulfa Zakiah*, Masnur Turnip
Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Tanjungpura, Jl. Prof. Dr. H. Hadawi Nawawi, Kota Pontianak, Kalimantan Barat 78124
*email: zulfazakiah@gmail.com

ABSTRACT

One of the most important stages in the process of transferring in vitro cultured seedlings to their natural environment is the acclimatization stage. The acclimatization stage is a critical period in plant propagation. The factors that most influence the growth of in vitro seedlings in the acclimatization process are suitable planting media and the administration of biostimulants. This study aimed to obtain the best planting medium and type of biostimulant for the growth of black orchid plantlets at the acclimatization stage. The study used a completely randomized design with a factorial pattern with two factors, namely first factor: type of growing media with 5 levels of treatment (wood sawdust, coconut coir, cocopeat, rice husk, sugarcane dregs) and second factor: type of biostimulant (without biostimulants; Moringa oleifera extract; Centella Asiatica extract; and Melastoma malabathricum extract). The results showed that the highest percentage of plantlet survival (100%) in the early stages of acclimatization was shown in plantlets grown on wood sawdust and coconut coir media. At the advanced acclimatization stage, the type of biostimulant significantly affected the parameters of survival percentage, tiller height, average number and width of leaves, and average number and length of roots. Optimal planting medium and biostimulant type for advanced acclimatization orchid growth is a single treatment of wood sawdust media with a tiller survival rate (100%), the highest number of leaves (4.65 strands), most roots (8.4 strands).

Keywords: *acclimatization, black orchid, biostimulant, plantlet, growth*

1. INTRODUCTION

Indonesia is one of the nations with the most extensive tropical forests and a great diversity of orchid species. Orchids are utilised as cut flowers and potted plants, which have evolved into a global flower industry. In addition, a number of orchid species are used as medicinal plants. The black orchid (Coelogyne pandurata Lindl.) is one of the natural orchids regarded as a defining characteristic of Indonesia.

This orchid is over-exploited due to its potential as an ornamental plant with a high economic value. Extinction will become a hazard if efforts to preserve black orchids through in vitro culture multiplication are not made to combat the exploitation of black orchids from nature.

Acclimatisation is the concluding step in tissue culture and is essential for the successful propagation of plants. Acclimatisation is an adaptation of the plantlet's environment from heterotrophic (in vitro) to autotrophic (in vivo) conditions and environmental conditions such as high light intensity, temperature, and low humidity (Sukmadijaya et al., 2013). According to Kasutjianingati et al. (2020), plantlets have a high mortality rate during acclimatisation because they must be able to absorb nutrients and perform photosynthesis flawlessly in vivo. While under these conditions, plant ability to assimilate nutrients remains low and transpiration rate is high.

Adi et al. (2014) found that growing medium factors and sowing techniques heavily influence the success of the acclimatisation process. An excellent growing medium promotes root development and provides adequate nutrients for plantlets. In addition, Hariyanto et al. (2019) explained that selecting the proper medium with low sensitivity, high aeration, permeability, and the correct acidity value is necessary to ensure the onset of plant development and growth. Wood charcoal and fern roots are typical mediums for orchid sowing (Ginting, 2008). However, the increasing demand for fern roots will result in a rise in price and a decrease in fern availability. Because the substrate is the foundation of orchid cultivation, it is necessary to locate alternative planting media suitable for orchid growth. Adi et al. (2014) reported that the acclimatisation of black orchids to media moss, ferns, and a combination of wood charcoal and coconut fibre resulted in a favourable growth response, while media containing wood charcoal exhibited weak growth. Hani et al. (2022) and Karmila et al. (2022) used wood ash as a growing medium to acclimate black orchid plantlets.

Biostimulants are an alternative cultivation technique that can stimulate plant growth. Biostimulants are non-fertilizer products that stimulate plant growth and vitality by enhancing the assimilation of nutrients and water. According to du Jardin (2015), secondary metabolites of plants can be utilised as a source of biostimulants, but little is known about their application. Bulgari et al. (2015) state that plant hormones, alkaloids, phenols, terpenoids, steroids, and saponins are biostimulants derived from secondary plant metabolites.

Secondary metabolites contained in various plant extracts can be used as a source of biostimulants. Culver et al. (2012) reported that applying crude extract of Moringa leaves (Moringa oleifera) to tomato plants through the leaves can increase the growth and production of tomatoes. Furthermore Singh et al. (2013) explained that the application of 12.5% moringa leaf extract on Pisum sativum significantly increased the wet weight (51.84%) and dry weight of the pods (67.29%). Research by Zakiah et al. (2017) showed that applying 25 mg/l crude extracts of gotu kola (Centella asiatica) could increase soybean plant height and leaf area. Biostimulant administration of gotu kola extract and moringa leaf extract on acclimatization of black orchid plantlets using sawdust media has been carried out but has not yielded significant results (Hani et al. (2022); Karmila et al. (2022). However, information regarding the use of several biostimulants for increasing the growth of black orchids on various growing media is still lacking.

2. MATERIAL AND METHODS

From June to October 2020, the research was conducted for five months at the Screening House, Department of Biology, FMIPA Untan, Pontianak.

Stationery, beaker glass, glass jars as maceration containers, plastic pots as planting containers, a hand sprayer, and cameras were used in this investigation. The materials used were 9-12 month-old black orchid plantlets (Coleogyne pandurata Lindl.) obtained from the Orchid Centre at the Pontianak Aloe Vera Centre, distilled water, gotu kola herb (Centella asiatica L.), Kelor leaves (Moringa oleifera), and cloves. (Melastoma malabathricum), 70% methanol and the growing medium used was coconut coir.

The investigation employs an experimental approach consisting of two stages:

1. Initial Acclimatisation Adaptation of plantlets in ex vitro conditions using five different varieties of growing media. At this stage, the percentage of plantlet growth in each medium up to one month of age is determined. weekly observations are taken.
2. At this juncture, plantlets are treated with biostimulants derived from extracts of three different plant species. This stage employs a wholly random factorial design, where factor A is the growing medium with five levels (wood sawdust, coconut fibre, cocopeat, rice husk, and sugarcane bagasse). Factor B was the type of biostimulant that consisted of four levels (without biostimulant/control, gotu kola extract, kelor leaf extract, and Cengkodok leaf extract); however, only one concentration, 25 mg/L, was used in this study (Zakiah et al., 2017). Each treatment was administered five times. Figure 1 depicts the work procedure in the form of a flowchart..

In this study, the following growth characteristics were observed and measured: percentage of plantlet survival (%), plantlet height (cm), number of tillers, number of leaves (strands), leaf length and girth (cm), number of roots and root length (cm).

All quantitative data in this investigation were statistically analysed using SPSS 19.0 and analysis of variance (ANOVA). If there were significant differences between regimens, a second test was conducted using Duncan's New Multiple Range Test (DNMRT) at a 5% significance level.

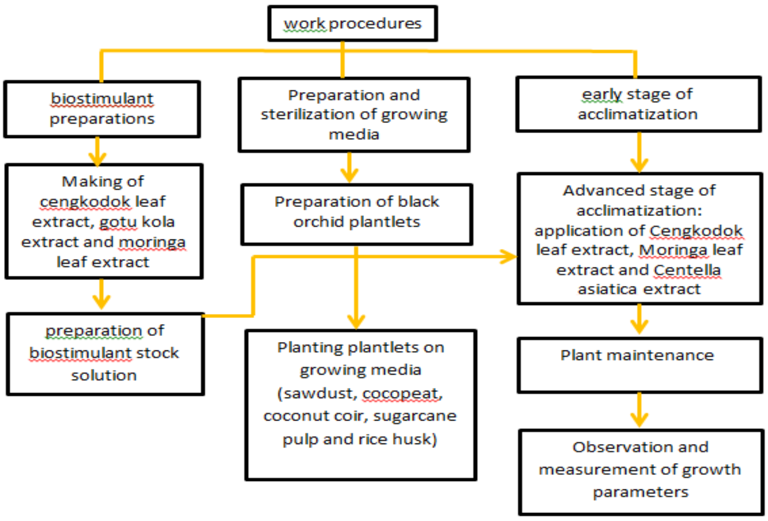

Figure 1. Work procedure flowchart

3. RESULT AND DISCUSSION

Based on the results of observations at the initial acclimatization and advanced acclimatization stages, the percentage of plantlet survival on various media is shown in Figure 2 and Figure 3.

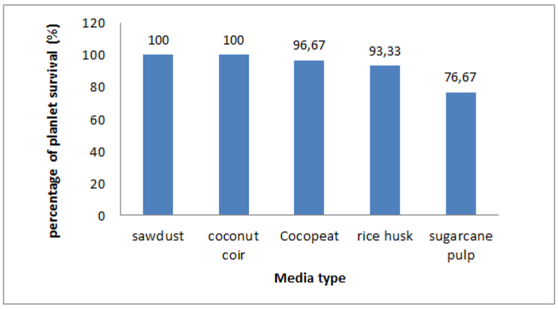

Figure 2. Percentage of survival of black orchid plantlets at the early acclimatization stage on some growing media without nutrition after 4 weeks of acclimatization to the hatch method.

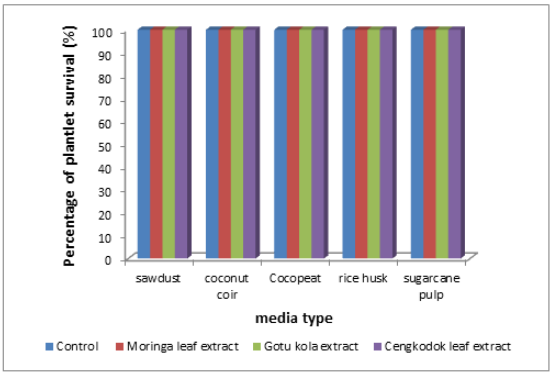

Figure 3. Percentage of survival of black orchid plantlets (%) acclimatized aged 12 weeks on various growing media after administration of moringa leaf extract, gotu kola extract and Cengkodok leaf extract using the lid method.

The survival rate of plantlets acclimatised to the lid method ranges from 76.67 to 100 percent, as shown in Figure 2. The survival rate of seedlings planted in sawdust and coconut fibre media was the highest, at one hundred percent. In contrast, plantlets cultivated in sugarcane dregs media exhibited the lowest percentage of life. Figure 3 demonstrates that all advanced acclimatisation plantlets can grow in all growing media using the lid method, with a 100% survival rate.

This demonstrates that the initial acclimatisation stage is a crucial phase in the growth of cultivated plants. In the early phases of acclimatisation, plantlets must adapt to their new environment, with each plantlet responding differently to environmental changes or the type of planting medium. Most plantlets survived in wood sawdust and coconut coir growing media. This demonstrates that both media have supported the initial growth of plantlets. Additionally, the water content of the planting medium has a significant impact on plantlet survival. According to Hanik et al. (2022), factors to consider when selecting a growing medium for orchid acclimatisation include air aeration, humidity, water holding capacity, level of fungal attack, and resistance to pests and diseases. This is because the growing medium serves as a location to retain moisture, store nutrients and water used to support plant growth. Furthermore, Febrianto et al. (2015) stated that good acclimatization media include having a high ability to hold water, good aeration to facilitate root growth and not easily overgrown with fungi. However, excess water content can cause plantlets to turn yellow, rot, and die quickly. Conversely, dry media or very low water content will cause plantlets to lack water for their growth.

When plantlets are transferred from aseptic (heterotrophic) conditions to autotrophic conditions, the plant tissue has not yet fully developed because the leaf cuticle layer is still very thin and has not yet fully formed. As a result, high temperatures or dry media conditions will inhibit growth because the plant tissue does not absorb enough water. Plantlets. Scaranari et al. (2009) state that tissue culture seedlings can wilt and die if they lose water through transpiration at the outset of acclimatisation. In addition, the ability of in vitro roots to acquire nutrients and water from the planting medium influences the viability of plantlets. In vitro roots cannot generally assimilate nutrients optimally, and some in vitro roots will perish. According to Nikmah et al. (2017), the roots generated by in vitro plantlets are typically few in number and weak, making them extremely fragile and incapable of functioning in vivo. In vitro roots will perish and be supplanted by later-formed new roots.

The variance analysis revealed that the biostimulant type significantly affected the average tiller height, average number of leaves, average leaf length, average leaf width, and black orchid root length. In contrast, the type of planting medium only affected the average number of leaves. The number of black orchid roots was substantially affected by the interaction between planting medium and biostimulant type (Tables 1 and 2).

Table 1. Results of the analysis of the diversity of growth of black orchid plantlets on several types of planting media after biostimulant treatment of several plant extracts

No.	Parameter	F Hit	CD (%)
1	Tiller height (cm)	$0,564^{ns}$	9,17
2	Number of puppies	$1,261^{ns}$	11,67
3	Number of leaves	$1,384^{ns}$	20,29
4	Leaf length(cm)	$0,798^{ns}$	10,41
5	Leaf width(cm)	$0,626^{ns}$	7,57
6	Number of roots	2,128*	34,45
7	root length(cm)	$1,119^{ns}$	23,68

Information: ns = no significant, (*) = significant, KK = Coefficient of Diversity (%)

Table 2. Growth of black orchid plantlets aged 12 weeks on several types of growing media after biostimulant treatment of several plant extracts

Treatment		Average						
Type of planting medium	Types of Biostimulants	Saplings height (cm)	Saplings number	Number of leaves (strands)	Leaf length (cm)	Leaf width (cm)	Number of roots	root length (cm)
Wood sawdust	No biostimulants	2,82 A	$2,00^{ns}$	4,20 A B	2,10 A	0,57 A	$3,80^{abc}$	1,78 A
	Moringa leaf extract	3,66 B	$2,40^{ns}$	4,60 B B	2,39 AB	0,63 B	$7,00^{defg}$	1,78 B
	Centella asiatica extract	3,49 B	$2,40^{ns}$	4,60 B B	2,57 AB	0,60 B	$5,80^{cdef}$	1,90 B
	Cengkodok leaf extract	3,80 B	$2,00^{ns}$	5,20 B B	3,09 B	0,71 B	$\mathbf{8,40^{g}}$	1,65 AB
coconut coir	No biostimulants	2,94 A	$1,80^{ns}$	3,60 A B	2,13 A	0,54 A	$5,20^{cdef}$	1,90 A
	Moringa leaf extract	3,36 B	$2,00^{ns}$	3,40 B B	2,48 AB	0,60 B	$5,00^{cde}$	1,89 B
	Centella asiatica extract	3,17 B	$2,20^{ns}$	4,80 B B	2.20 AB	0,63 B	$6,40^{defg}$	1,62 B
	Cengkodok leaf extract	3,47 B	$1,80^{ns}$	4,20 B B	2,36 B	0,62 B	$7,80^{fg}$	1,60 AB

Cocopeat	No biostimulants	2,88 A	$2,20^{ns}$	3,40 A AB	2,49 A	0,53 A	$4,80^{bcde}$	1,21 A
	Moringa leaf extract	3,62 B	$1,80^{ns}$	4,80 B AB	2,42 AB	0,64 B	$7,60^{efg}$	1,40 B
	Centella asiatica extract	3,43 B	$2,60^{ns}$	5,00 B AB	2,24 AB	0,59 B	$3,80^{abc}$	1,75 B
	Cengkodok leaf extract	3,55 B	$2,40^{ns}$	3,60 B AB	2,69 B	0,56 B	$6,80^{defg}$	1,78 AB
Rice husk	No biostimulants	3,00 A	$2,00^{ns}$	3,00 A A	2,19 A	0,53 A	$2,20^{a}$	0,93 A
	Moringa leaf extract	3,03 B	$2,40^{ns}$	3,40 B A	2,46 AB	0,61 B	$5,20^{cdef}$	1,72 B
	Centella asiatica extract	3,54 B	$1,80^{ns}$	3,20 B A	2,34 AB	0,63 B	$5,00^{cdef}$	2,00 B
	Cengkodok leaf extract	3,06 B	$2,00^{ns}$	3,60 B A	2,49 B	0,56 B	$1,80^{a}$	1,21 AB
Sugarcane waste	No biostimulants	2,88 A	$1,60^{ns}$	2,40 A B	1,92 A	0,54 A	$2,60^{ab}$	0,91 A
	Moringa leaf extract	3,15 B	$2,40^{ns}$	4,60 B B	2,44 AB	0,58 B	$5,80^{cdef}$	1,80 B
	Centella asiatica extract	3,15 B	$2,20^{ns}$	5,40 B B	2,52 AB	0,61 B	$7,20^{defg}$	2,55 B
	Cengkodok leaf extract	3,43 B	$2,20^{ns}$	4,80 B B	2,21 B	0,62 B	$6,20^{cdefg}$	1,93 AB

Information:
Ns = no significant
Numbers followed by different uppercase letters in the rows and columns show significantly different results on the single factor of the media type and biostimulant type. In contrast, numbers followed by different lowercase letters in the rows and columns show significantly different results on the interaction factor of the media type and type of biostimulant based on Duncan's advanced test at a 95% confidence level.

The percentage of plantlets that survived to an advanced stage indicated that the variety of growing medium used had no discernible effect on the viability of plantlets. This study utilised sawdust, cocopeat, sugarcane residues, coconut coir, and rice husks, all of which had a comparable capacity to absorb water. This satisfies the media requirements for the acclimatisation procedure, as described by Kurniasih et al. (2017). In the acclimatisation stage, orchid growing media should not easily rot and decompose, not be a source of disease, have good aeration and drainage, be able to bind water and nutrients optimally, be able to retain moisture around the roots, and be environmentally friendly, readily available, and inexpensive.

The high survival rate is also influenced by "Gandasil D" foliar fertiliser that is sprayed every three days on the underside of the foliage. When fertiliser is administered through the leaves, it is rapidly absorbed by the plant and immediately impacts plant growth. In addition, the foliar fertiliser Gandasil's nutrient content in the form of N (20%), P (15%), K (15%) and additional microelements Mg, Mn, B, Cu, Co, and Zn will promote organ development. vegetative vegetation. According to Agustina (2004) in (Andalasari et al., 2014), fertiliser applied to the leaves diffuses into the leaf tissue through the stomata and enters the chloroplast cells in guard cells, leaf mesophyll and vascular sheaths, where it participates in photosynthesis.

The administration of biostimulants in the form of gotu kola extract, kelor leaf extract, and Senduduk leaf extract will increase the growth and viability of orchid plantlets. The amount of secondary metabolites present in each extract influences the growth of orchids after administration of the three extracts. Phytochemical studies revealed that the three extracts contained phenolic compounds, steroids (gotu kola and moringa leaves), and triterpenoids (Senduduk leaves) as a result of qualitative analysis. This demonstrates that terpenoids, steroids, and phenolics cooperate with secondary metabolites such as steroids, flavonoids, saponins, and tannins to promote plant growth. Low concentrations of these three compounds are known to function as growth regulators. According to Ertani et al. (2016), certain phenolic compounds serve as plant growth rate regulators because they can increase the rate of carbohydrate and nitrogen metabolism in plant tissues.

The formation of new leaves will determine the number of leaves produced throughout the sowing period. The sole application of biostimulant and media significantly impacted the number of black orchid leaves. The solitary Centella asiatica extract treatment yielded the greatest average number of leaves, 4.60 (Table 2). The average number of leaves differed significantly from the control treatment but not significantly from the Moringa leaf extract and Senduduk leaf extract treatments. This indicates that the secondary metabolite content of each of the applied extracts influences cell differentiation in shoot meristems during leaf formation. These results also indicate that the three extracts containing terpenoids, steroids, and phenolic compounds increase plant growth by producing growth hormone-like activities. According to Zi et al. (2014), terpenoid compounds in plant tissues can serve as a precursor of the bioactive diterpenoid gibberellin or as a precursor of other terpenoid compounds that can stimulate the action of gibberellins. The results of this study differed from those of Hani et al. (2022), who used gotu kola extract as a biostimulant and sawdust as a planting medium and found that gotu kola extract concentrations did not impact all observed growth parameters.

The average number of leaves is also affected by the sole treatment of the type of growing medium, as shown in Table 2. The medium that produced the greatest average number of leaves was debris, with an average of 4.65. The average number of leaves was substantially different on media containing rice husk but not on other media. The high number of leaves in sawdust and other media suggested that the four media were suitable for the growth of orchid plantlets, particularly in their capacity to retain water, aeration, and drainage. The high number of leaves in sawdust media is also associated with the high number of roots in this treatment, particularly in the interaction treatment of wood sawdust media and Senduduk leaf extract. The number of roots in all media except rice husk combined with senduduk leaf extract did not differ significantly from sawdust (Table 2). Media with excellent aeration and drainage and extract secondary metabolites with plant growth regulator activity strongly influence root development. Many leaves will increase photosynthesis, and the resulting energy will promote root development.

A single biostimulant treatment substantially affected tiller height, leaf width, and root length. The mean tiller height, leaf width, and root length in the three biostimulant treatments were substantially different from the control but not from one another. This demonstrates that the amount of secondary metabolites in each extract affects the increase in tiller height, leaf girth, and root length. The three extracts contain a group of compounds that interact synergistically to stimulate cell division, elongation, or expansion. The group of compounds believed to increase tiller height, leaf width, and root length consists of steroid and triterpenoid compounds that play a role in the biosynthesis of the diterpenoid compound gibberellin. According to Pichersky & Raguso (2018), terpenoid and steroid compounds that enter plant tissue may be used to form growth hormones such as gibberellins, auxins and cytokinins. Furthermore, Rafiee et al. (2016) explained that the applied biostimulants will affect plant metabolism such as stimulating synthesis and increasing the activity of phytohormones, facilitating the uptake of nutrients from the substrate and stimulating root growth. The growth of orchid tillers in each treatment is shown in Figure 4.

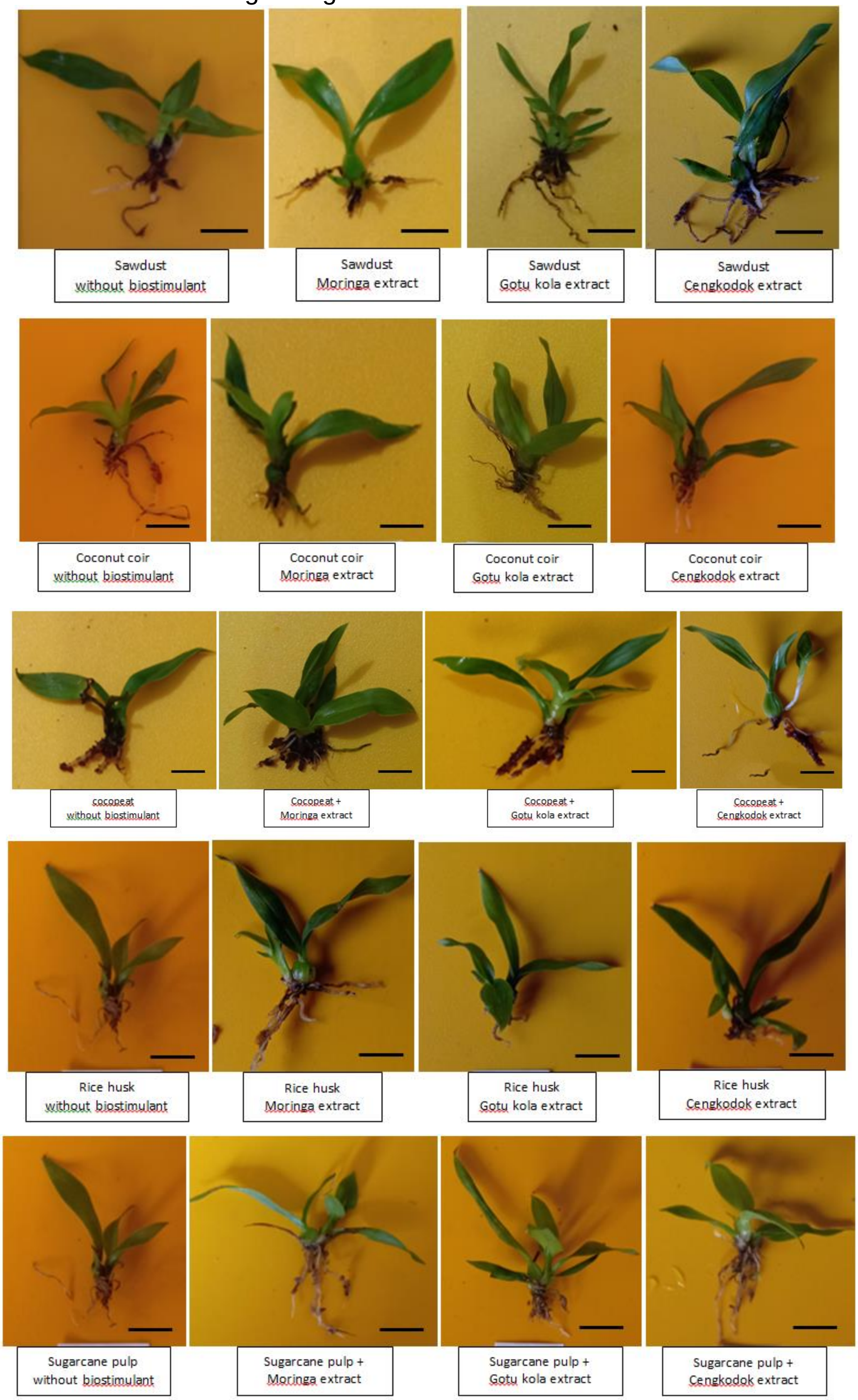

Figure 4. Comparison of the results of observations on the growth of black orchid plantlets on growth media and biostimulant treatments at different ages of 12 weeks Bar scale = 1 cm

4. CONCLUSION

The optimal medium for the growth of orchids during the acclimatisation phase is wood sawdust media, which generates the highest percentage of living organisms (100%) and the greatest number of roots (8.4 roots). The biostimulant type significantly affects the parameters tiller height, average number and diameter of leaves, and average number and length of roots, with Senduduk leaf extract being the most effective biostimulant.

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