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# Effects of Organic Growing Media on Growth, Yield and Bioactive Compound of Black Ginger (*Kaempferia parviflora*) Cultivated using Soilless Culture

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The experiment, conducted under a side-netted rain shelter, holds promise for the future of black ginger cultivation. Five mixtures of organic growing media were evaluated: 100% coco peat; 100% rice husk ash; 70% coco peat + 30% rice husk ash; 30% coco peat + 70% rice husk ash; and 50% coco peat + 50% rice husk ash. The black ginger rhizomes were harvested eight months after planting. The plants grown in 50% coco peat + 50% rice husk ash mixtures showed the best growth performance and yield, producing the highest vegetative fresh weight shoot height (678 g) and rhizome yield (582 g per plant). The lowest rhizome yield (154 g) was obtained from plants planted in 100% coco peat. However, plants cultivated in 100% coco peat gave rise to the highest 4,5,7-trimethoxyflavone compared to the other samples. Therefore, the black ginger plants cultivated in 50% coco peat + 50% rice husk ash mixtures growing media using a soilless culture system

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demonstrated the best plant growth and yields. However, 100% coco peat growing media should be considered to achieve the highest 4,5,7-trimethoxyflavone accumulation in black ginger rhizomes, opening up new possibilities for the future of black ginger cultivation.

*Keywords: black ginger; soilless culture system; growing media; coco peat; rice husk ash.*

## 1. INTRODUCTION

Black ginger, scientifically known as *Kaempferia parviflora*, is a ginger plant that belongs to the tropical and sub-tropical Zingiberaceae family. It is native to Southeast Asia and is a perennial plant with thick dark purple tuberous roots or rhizomes. This plant has been cultivated for use as a spice and for herbal medicine. The leaves of black ginger are approximately 6 to 8 cm long, oblong in shape and the plant produces purple and white flowers [1]. Black ginger is widely used in alternative medicine to treat various ailments, including fungal infections, gastrointestinal disorders, decreased vitality, allergies, body pains, oral diseases, and male impotence, as well as for overall health promotion [2,3].

A soilless culture system is a method of growing plants that replicates the functions of soil by physically supporting the plant and providing a rooting environment with optimal levels of water and nutrients. The yields of chillies, rock melons, and tomatoes cultivated in soilless systems have increased by 3 to 5 times compared to those grown using conventional soil methods [4,5]. In these soilless production systems, various types of growing media, such as rockwool, perlite, vermiculite, and peat, are used for cultivating a wide range of crops [6,7,8]. However, media like rockwool, perlite, and vermiculite are costly due to the need for importation. Therefore, it is advisable to use alternative, more affordable, and locally available growing media, such as coconut fibers and rice husk ash [9]. One of the crucial factors affecting plant fertility, aside from water and nutrient content, is soil aeration [10]. Different plant species have varied rooting systems, enabling them to grow under diverse oxygen conditions [11].

Several studies have examined the physical properties of growth media, including available water capacity (AWC) and air-filled porosity (AFP) [12,13,14,15]. AWC measures the water content within the media, while AFP assesses the level of oxygen availability or aeration [16]. According to Humara et al. [17], excessive water content in growing media can decrease AFP and aeration, resulting in waterlogging and hypoxia, which are harmful to most plant species. Ensuring an appropriate water level in growing media is essential for optimal plant growth and development [18].

Phytochemical studies revealed that black ginger's rhizomes contain phenolic and flavonoid compounds including flavones, flavanones and chalcones [19,20]. The rhizomes of K. parviflora contain a variety of methoxyflavones, which contribute to its medicinal potential [19]. Among these methoxyflavones, 4,5,7-trimethoxyflavone stands out as a representative compound with a wide range of pharmacological properties [3]. These make 4,5,7-trimethoxyflavone a promising candidate for developing therapeutic agents targeting various health conditions, including inflammation, oxidative stress, cancer, microbial infections, cardiovascular diseases, and neurodegenerative disorders [21]. Domestic demand for black ginger is high and has increased significantly as people become more interested in its medicinal properties. However, the demand for black ginger rhizomes in Malaysia can hardly be fulfilled due to the low production yield and planting materials [22]. Therefore, cultivating ginger using a soilless culture system could be an alternative method to increase rhizome yields and address the supply shortage problem.

The potential to enhance the growth and yield of black ginger rhizomes using a soilless system is supported by significant yield increases observed in chillies, rock melons, tomatoes, and other leafy and fruity vegetables grown on various media [4,5]. Consequently, this study aims to evaluate the effects of soilless growing media, such as coco peat and rice husk ash, on the growth and yield of black ginger. The primary objective is to identify the optimal growing media for cultivating black ginger using a soilless culture system.

## 2. MATERIALS AND METHODS

### 2.1 Planting Materials

Black ginger is propagated by planting 8-month-old rhizomes**.** These rhizomes are cut into 4 cm pieces, each weighing about 30 g and containing

2-3 growth buds. Before planting, the rhizome sections are treated with propamocarb. New shoots emerge approximately 2-3 weeks after planting. Mature rhizomes are ready for harvest after 8 months of growth.

### 2.2 Study Area

The study was conducted in a 30-meter long, 10-meter wide, and 4.5-meter-high rain shelter located at the MARDI Station in Serdang, Selangor, Malaysia. This structure was built with a galvanized steel frame, covered with transparent polyethylene film for the roof and insect-proof netting on the sides. To prevent insect entry, the shelter had double doors.

### 2.3 Treatments and Experimental Design

The experiment used a randomized complete block design (RCBD) with five different growing media treatments and three replicates. Each treatment consisted of 30 black ginger plants. The growing media were mixtures of coco peat and rice husk ash in varying proportions: T1: 100% coco peat; T2: 100% rice husk ash; T3: 70% coco peat + 30% rice husk ash; T4: 30% coco peat + 70% rice husk ash and T5: 50% coco peat + 50% rice husk ash. Each polyethene bags were filled with four kilograms of growing media and the treatments were mixed accordingly to obtain the weight. The prepared growing media were filled into 16 cm x 16 cm black polyethylene bags. Black ginger rhizomes were then planted in these bags. The bags were placed on irrigation lines with a spacing of 30 cm x 150 cm under the rain shelter. Each plant received nutrient solution through a dripper placed on the growing medium surface.

### 2.4 Irrigation set-up

The irrigation system consisted of a 1500-liter water tank, a 1.5 horsepower pump, a filter, a pressure gauge, and four looped lateral lines (28 m long each) within the rain shelter. Each line held 100 drippers spaced 30 cm apart, delivering water to plants in 16x16 cm black polyethylene bags arranged in rows 1.5 m apart. Valves controlled water flow through the lateral lines, while 0.3 m long micro tubes and arrow drippers supplied nutrient solutions to the plants.

### 2.5 Fertilizer Preparation and Irrigation Frequencies

MARDI developed a water-soluble fertilizer specifically tailored to the nutritional needs of black ginger rhizomes. The fertilizer formulation followed the guidelines outlined by Yaseer Suhaimi et al. [23]. The fertilizer was divided into two main components: stock solution A: containing calcium nitrate and iron and stock solution B: containing all other necessary macro and micronutrients. Both solutions were prepared at a 100x concentration for later dilution. To ensure complete dissolution, each component was added individually to water and stirred before combining with other ingredients. The final solutions were stored in 100-liter containers.

The irrigation solutions were prepared in a 1,500-litre tank by combining Stock A and Stock B in a 1:1 ratio until the desired electrical conductivity (EC) of 1800-2400 µS was achieved. Irrigation was automated using a digital timer, with schedules varying throughout the growth period. In the first 3 months, plants were watered twice daily (0800h and 1600h) with 500 ml per day. From the 4th to 7th month, irrigation increased to three times daily (0800h, 1000h, and 1600h) with 750 ml per day. In the final month, watering reduced to once daily (0800h) with 250 ml per day. Each irrigation session lasted 3 minutes, delivering an equal amount of fertilizer solution to all polyethylene bags. Standard horticultural practices were followed for plant care, including biweekly applications of malathion (insecticide) and benlate (fungicide) for pest and disease control.

### 2.6 Parameters Measurements

The growth of black ginger plants was monitored through monthly measurements of various parameters. Plant height was recorded, and the weights of leaves, shoots, and rhizomes were measured using randomly selected plants. After an eight-month growing period, the rhizomes were harvested to evaluate their growth, yield, and bioactive compound content. To ensure accuracy and prevent water loss through desiccation, the rhizome weights were measured immediately following harvest. This systematic approach to data collection allowed for a comprehensive assessment of the plants' development throughout their growth cycle and at the time of harvest.

### 2.7 Air-filled Porosity (AFP) and Container Moisture Capacity (CMC)

The container moisture capacity (CMC) represents the amount of water present in the growing media after saturation and subsequent drainage. The CMC of five different media mixtures was measured at two different time intervals using the formula: (saturated mass–dry mass)/dry volume. Measurements were taken one month after planting by weighing the containers at 1 hour and 5 hours after watering. Air-filled porosity (AFP), also known as air capacity, is a measure of the volume proportion that contains air in a medium after it has been saturated with water and allowed to drain. This parameter is crucial for understanding the aeration properties of the growing medium. The measurement of AFP in this study followed the methodology outlined by Bunt [24], ensuring a standardized approach to quantifying this important characteristic of the substrate. This measurement provides insights into the medium's ability to maintain adequate oxygen levels for root respiration and overall plant health.

### 2.8 Plant Materials and Preparation of Extracts

Rhizome samples were obtained and washed with running tap water to remove surface pollutants and cut into thin slices. They were then dried under hot air oven at 60 °C for 48 h. After drying (moisture content of 8-10% dry basis), the samples were ground into a fine powder and kept in an air-tight container before extraction. The samples were extracted with 70% methanol (1:10) under sonication for 1 h. The samples were centrifuged at 5,000 rpm for 10 minutes to separate the supernatant from the sediment. The extraction process was conducted in triplicate under consistent conditions to ensure thorough and reliable results. After each extraction, the resulting filtrates were pooled together. This combined solution was then subjected to rotary evaporation, a process that removed all liquid and yielded the crude extracts in dry form. To preserve samples integrity and prevent degradation, these crude extracts were stored at a low temperature of 4°C until they were needed for subsequent analyses and determinations.

### 2.9 Identification and Quantification of 4,5,7-Trimethoxyflavone

Before analysis, the methanolic crude extracts were filtered through a 0.22 µm pore size nylon membrane filter. Identification of 4,5,7-trimethoxyflavone was performed on high-performance liquid chromatography (HPLC). The compound was chromatographically separated using a XBRIDGE (150 mm x 4.6 mm x 3 µm) column and maintained at 40 °C. A linear binary gradient of water (0.1% formic acid) and acetonitrile (0.1% formic acid) was used as mobile phases A and B, respectively. The flow rate was set at 1 mL/min and the injection volume was 1 µL. The UV–vis absorption chromatogram was detected at 265 nm using a DAD detector. The quantification of 4,5,7-trimethoxyflavone in the extracts was performed using a calibration curve method. This approach involved creating a calibration curve by plotting the peak areas of known concentrations of a 4,5,7-trimethoxyflavone standard. From this curve, a regression equation was derived, establishing a relationship between peak area and concentration. The peak area of 4,5,7-trimethoxyflavone in the sample extracts was then measured and applied to this regression equation.

### 2.10 Statistical Analysis

The data obtained were subjected to statistical analysis using analysis of variance (ANOVA) procedures to test the significant effects of all the variables investigated, employing SAS version 9.1. Means were separated using the Duncan Multiple Range Test (DMRT) to determine significance at $p<0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1 Air-filled Porosity (AFP) and Container Moisture Capacity (CMC)

The 100% coco peat treatment had the highest porosity after both 1 hour and 5 hours of irrigation (Table 1). There were no significant differences in the air-filled porosity (AFP) values between the 50% coco peat and 50% rice husk ash mixture and the 70% coco peat and 30% rice husk ash mixture. The 100% rice husk ash treatment had the second lowest initial (6.7%) and final (9.4%) porosity at both time intervals after irrigation, followed by the 30% coco peat and 70% rice husk ash mixture (initial: 5.8%, final: 7.8%). The AFP values from 100% coco peat and mixtures with higher coco peat content (up to 70%) increased compared to 100% rice husk ash and mixtures with higher rice husk ash content (up to 70%). Mixtures with a high content of rice husk ash had lower AFP values due to their compaction and high water retention properties. Adding coco peat to rice husk ash increased the air volume, enhancing air capacity and reducing water content in the mixtures. Air availability in the growing media is a crucial factor for successful plant growth in containers [25]. Container moisture capacity (CMC) measures the water availability or content in the growing media, and its values decreased 5 hours after irrigation (Table 1). The highest initial and final CMC values were observed in the mixture of 30% coco peat and 70% rice husk ash, followed by 100% rice husk ash, the mixture of 50% coco peat and 50% rice husk ash, the mixture of 70% coco peat and 30% rice husk ash, with the lowest CMC in the 100% coco peat growing media. The differences in CMC values between 100% rice husk ash and 100% coco peat were 26.7% and 22.2%, respectively, at both time intervals after irrigation. These results indicated that adding rice husk ash to coco peat increased the moisture content while decreasing the AFP of the growing media. Air retention and moisture content in the growing media are essential for optimal plant growth in containers [25].

### 3.2 Effects on Plant Growth

There were significant differences in vegetative fresh weight between treatments at $p \leq 0.05$ (Table 2). The highest vegetative fresh weight was produced by black ginger cultivated in 50% coco peat and 50% rice husk ash mixtures with an average weight of 678 g and the lowest were those cultivated in 100% coco peat with an average weight of 495 g. This could be due to the moderate porosity of 50% coco peat and 50% rice husk ash mixtures that can retain suitable moisture in the growing media compared to the other treatments. Coco peat as growing media has a higher porosity compared to rice husk ash, which has low porosity. Porosity characteristics allow the growing media to retain moisture and create air-filled space in the growing media. Combining two media types alters the media characteristics, as seen in the study. This higher porosity property drained the excess fertiliser solution between the irrigation schedules more quickly. The mixtures of coco peat and rice husk ash could have increased the water-holding capacity and maintained the moisture that is needed for rhizome growth.

Plants cultivated in 30% coco peat and 70% rice husk ash mixtures showed the highest plant height, number of tillers and SPAD value compared to other treatments. However, there were no significant differences in plant height and tiller diameter between treatments. The type of media used to cultivate the black ginger plant did not affect these two parameters. Previous studies showed that higher content of rice husk ash in the medium added more moisture content that lowered dissolved oxygen in the media, consequently reducing the ginger plant's height compared to 100% coco peat [26]. Other studies also showed that high water holding capacity reduces tomato and cucumber growth and yield [27,28]. The growth requirements of the black ginger plant differ from those of other rhizomatic plant species because the plant does not require a high water content to grow [29]. High water content conditions might increase the chances of plant pathogens affecting the rhizomes in container cultivation [30,31].

### 3.3 Effects on Rhizome Yield

For commercial purposes, black ginger rhizomes are typically harvested eight months after sowing. In this study, the rhizomes were harvested after eight months, and their fresh weight was measured. The interior flesh and epidermis of the rhizomes were darker purple compared to the mother seed piece, and they produced a rancid odor. Significant differences in rhizome yield were observed between the different treatments after eight months of cultivation (Table 2). The highest average fresh rhizome yield was obtained from plants cultivated in a mixture of 50% coco peat and 50% rice husk ash, followed by mixtures of 70% coco peat and 30% rice husk ash, 30% coco peat and 70% rice husk ash, 100% rice husk ash, and finally, 100% coco peat. These results indicated that black ginger cultivated in an equal mixture of coco peat and rice husk ash increased the rhizome yield by up to 105% compared to those grown in media containing 100% coco peat.

Moderate moisture content between irrigation in the 50% coco peat and 50% rice husk ash supported the underground rhizomes for growth. The black ginger plant did not require too much moisture as it is detrimental to rhizome growth. Meanwhile, too low moisture in the root zone created a dry condition that stunted rhizome growth. A 50:50 ratio of coco peat and rice husk ash have a strong capillarity that provides more uniform moisture conditions for black ginger roots. For crops grown in containers, it is important to consider the tendency of most root systems to grow gravitropically to form a dense layer at the bottom of the containers [32]. These conditions can increase aeration in the base mix and reduce surface drying by lifting the moisture higher up in the polyethene bags. This increases the volume of the mix suitable for root development and improves access to moisture and fertiliser. This moisture redistribution is possibly one of the reasons plants grown in mixtures of 50% coco peat and 50% rice husk ash have higher rhizome yields. Aeration in the growing medium is positively related to AFP and negatively to water content [33]. The 50% coco peat and 50% rice husk ash mixtures are less acidic with a pH suitable to facilitate ginger growth, allowing the plant roots to absorb nutrients efficiently.

**Table 1. Physical properties of growing media at 2 different times after irrigation**

| Treatment | Air-filled porosity (%) | | Container moisture capacity (%) | |
|---|---|---|---|---|
| | 1 h | 5 h | 1 h | 5 h |
| 100% CP | 9.8$^a$ | 14.0$^a$ | 41.5$^e$ | 36.8$^e$ |
| 100% RHA | 6.7$^c$ | 9.4$^c$ | 68.2$^b$ | 59.0$^b$ |
| 70% CP + 30% RHA | 8.4$^b$ | 10.3$^b$ | 51.0$^d$ | 47.3$^d$ |
| 30% CP + 70% RHA | 5.8$^d$ | 7.8$^d$ | 71.4$^a$ | 67.6$^a$ |
| 50% CP + 50% RHA | 8.8$^b$ | 10.4$^b$ | 55.5$^c$ | 52.5$^c$ |

*Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$*
*CP = Coco peat; RHA = Rice husk ash*

**Table 2. Plant growth and rhizome yield after eight months of cultivation**

| Treatment | Plant height (cm) | Vegetative fresh weight (g) | Number of tillers | SPAD value | Diameter of tiller (cm) | Average Fresh rhizome yield per plant (g) | Rhizome-to-shoot ratio |
|---|---|---|---|---|---|---|---|
| 100% CP | 58$^a$ | 495$^e$ | 24$^a$ | 42$^d$ | 0.12$^a$ | 154$^e$ | 0.32$^e$ |
| 100% RHA | 60$^a$ | 582$^c$ | 22$^b$ | 48$^c$ | 0.14$^a$ | 228$^d$ | 0.39$^d$ |
| 70% CP + 30% RHA | 56$^a$ | 620$^b$ | 18$^c$ | 52$^b$ | 0.12$^a$ | 418$^b$ | 0.68$^b$ |
| 30% CP + 70% RHA | 59$^a$ | 548$^d$ | 25$^a$ | 58$^a$ | 0.14$^a$ | 298$^c$ | 0.55$^c$ |
| 50% CP + 50% RHA | 58$^a$ | 678$^a$ | 22$^b$ | 50$^b$ | 0.13$^a$ | 582$^a$ | 0.86$^a$ |

*Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$*
*CP = Coco peat; RHA = Rice husk ash*

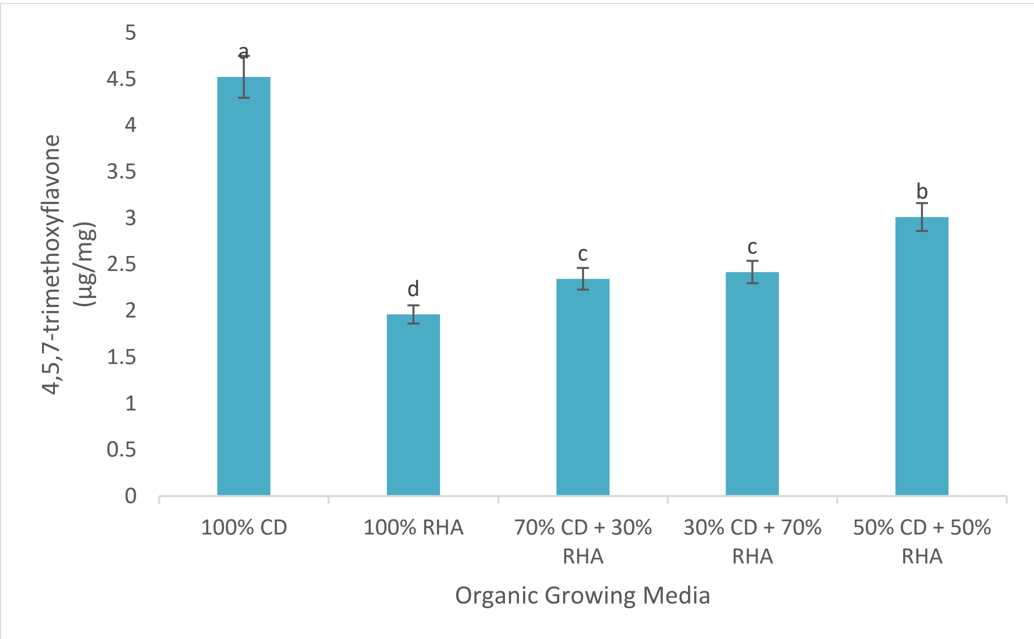


**Fig. 1. Effect of growing media on 4,5,7-trimethoxyflavone accumulation in the black ginger rhizomes after eight months of cultivation**

Media with a high content of coco peat resulted in lower rhizome yields throughout the cultivation period, with 100% coco peat exhibiting the lowest yield. These findings are consistent with a study by Wan Zaliha and Nurul Azila [34], which reported a significant decrease in black ginger rhizome yield when grown in 100% coco peat. Additionally, a study by Hayden et al. [35] found that rhizome growth depends on the type of medium used. The growing medium acts as a heat insulator, providing the necessary warmth to enhance rhizome growth. The overall biomass of black ginger plants can be divided into aboveground biomass (leaves and stems) and underground biomass (rhizomes and roots). This study showed significant differences between treatments in the rhizome-to-shoot ratio. The ratio of underground biomass to aboveground biomass was highest in plants cultivated in a mixture of 50% coco peat and 50% rice husk ash, with a ratio of 0.86 (Table 2). All treatments showed higher underground biomass compared to aboveground biomass. The high ratio of underground biomass to aboveground biomass indicates that the roots effectively supply the top of the plant with water, nutrients, stored carbohydrates, and certain growth regulators [36].

### 3.4 Bioactive Compound

Fig. 1 showed that, black ginger rhizomes obtained from 100% coco peat contained the highest 4,5,7-trimethoxyflavone compared to the other samples. Conversely, the 100% rice husk ash sample gave the lowest values for 4,5,7-trimethoxyflavone. The various mixtures of coco peat and rice husk ash yield intermediate values, but none surpass the 100% coco peat sample for 4,5,7-trimethoxyflavone accumulation in the rhizomes. The study reveals that the growing media composition significantly affects the 4,5,7-trimethoxyflavone content in black ginger rhizomes. Among the tested media, the rhizomes grown in 100% coco peat exhibited the highest content of 4,5,7-trimethoxyflavone at 4.521 µg/mg. This indicates that as a growth medium, coco peat provides a favourable environment for synthesizing or accumulating this flavone. The specific properties of coco peat, such as its low water retention and high aeration, might contribute to the enhanced production of 4,5,7-trimethoxyflavone [37]. However, black ginger plant cultivated using 100% of coco peat gave the lowest rhizomes yield compared to other treatments.

In contrast, the rhizomes grown in 100% rice husk ash showed the lowest 4,5,7-trimethoxyflavone content at 1.959 µg/mg. This suggests that rice husk ash, due to its physical structure, high moisture, and low aeration, is less conducive to the production of 4,5,7-trimethoxyflavone in black ginger rhizomes. Mixtures of coco peat and rice husk ash resulted in intermediate levels of 4,5,7-trimethoxyflavone, with the mixture of 50% coco peat + 50% rice husk ash mixture yielding a relatively higher content than other mixtures but still lower than 100% coco peat. Similar data presented in previous studies indicate that the fruit yield per plant, fruit weight, firmness, salinity, total soluble sugar (TSS) and the anthocyanin and phenolic contents were higher in plants grown in coir fibre than in plants grown in soil [38]. Therefore, the media composition plays a crucial role in the biosynthesis of essential compounds in black ginger, and optimising this can enhance the medicinal and commercial value of the rhizomes.

## 4. CONCLUSION

The mixture of coco peat and rice husk ash significantly alters the characteristics of growing media that affected plant height, vegetative fresh weight, number of tillers, SPAD value, diameter of tiller, average fresh rhizome yield per plant, rhizome-to-shoot ratio and 4,5,7-trimethoxyflavone. Media containing 50% coco peat and 50% rice husk ash mixtures showed good growth and increased the rhizome yield up to 105% compared to those containing high coco peat. It can be concluded that 50% coco peat and 50% rice husk ash mixtures are the best media for growing black ginger in the soilless culture system. However, 100% coco peat was recommended for high 4,5,7-trimethoxyflavone accumulation in the black ginger rhizome.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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