# Effects of organic substrates on growth, yield and bioactive compound of black ginger (*Kaempferia parviflora*) cultivated using soilless culture


## ABSTRACT

The study, conducted under a side-netted rain shelter, holds promise for the future of black ginger cultivation. Five combinations of growth substrates were evaluated: 100% coco peat; 100% burnt paddy husks; 70% coco peat + 30% burnt paddy husks; 30% coco peat + 70% burnt paddy husks; and 50% coco peat + 50% burnt paddy husks. The black ginger plants were randomly selected, and the rhizomes were harvested eight months after sowing. The plants grown in 50% coco peat + 50% burnt paddy husks 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% burnt paddy husks mixtures substrate using a soilless culture system demonstrated the best plant growth and yields. However, 100% coco peat substrates 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, soilless substrate, coco peat, burnt paddy husks.*




## 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 as an alternative medicine in treating various types of diseases including fungal infections, gastrointestinal disorders, decreased vitality, reduced allergies, promoting health, relieving body pains, oral disease, gastrointestinal disorders, and rectifying male impotence [2,3].

Soilless culture system is a method of growing plants that provides the same functions as the soil by supporting the plant physically while providing a rooting environment that gives access to optimum levels of water and nutrients. The yields of chillies, rock melons and tomatoes cultivated in soilless system increased 3 – 5 times compared to those using conventional method [4, 5]. In soilless production system, many types of growing media or substrates such as rockwool, perlite, vermiculite and peat have been used to grow many kinds of crops [6, 7, 8]. Media such as rockwool, perlite and vermiculite are expensive because they have to be imported. Hence, alternative substrates that are cheaper and locally available such as coconut fibers and burnt paddy husks should be used as alternative media [9]. One of the most important factors influencing plant fertility, besides water and nutrient content, is soil aeration [10]. Plant species have different rooting systems, enabling them to grow under different oxygen requirements [11].

Several studies have been conducted to analyse the physical properties of growth media including available water capacity (AWC) and air-filled porosity (AFP) [12, 13, 14,15]. AWC indicates the water content of substrates and AFP gives the estimation of oxygen availability or level of aeration in the substrates [16]. According to Humara et al. (2002), high water content in the growing substrates can reduce AFP and aeration, leading to logging and hypoxia which are detrimental to most plant species. A sufficient amount of water in growing substrates is one of the most critical factors for 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 soilless culture system could be an alternative method for increasing rhizome yields to overcome the supply shortage problem.

There is potential to increase the growth and yield of black ginger rhizomes using soilless system based on significant increase in yields of chillies, rock melons, tomatoes, and other leafy and fruity vegetables grown on various media [4, 5]. Thus, this study was conducted to determine the effects of soilless substrates such as coco peat and burnt paddy husks on growth and yield of black ginger. The main objective was to determine the optimum growth substrate for black ginger cultivation using soilless culture system.

## 2. MATERIAL AND METHODS

### 2.1 Planting materials

Black ginger is vegetatively propagated using 8-month-old rhizomes, and the shoot appears 2 - 3 weeks after sowing. Each of the rhizomes was cut into smaller pieces about 4 cm long and 30 g in weight. Each of the seed rhizomes contained 2 – 3 point buds. The seed rhizomes were treated with previcur-N prior to planting. Black ginger can be harvested at 8 months as mature rhizomes.

### 2.2 Study area

The study used a side-netted rain shelter 30 m long x 10 m wide x 4.5 m high located in MARDI Station, Serdang, Selangor, Malaysia. All structures were made of galvanised steel frames with transparent polyethylene film (180 µm thick) roofing and insect repellent net (0.1 x 0.1 $mm^2$) side cladding. Entrance into the shelter must be through double doors to reduce the chance of insects entering.

## 2.3 Treatments and experimental design

The treatments were arranged in a randomised complete block design (RCBD) with five levels of treatment with three replicates and 30 plants per treatment. The coco peat and burnt paddy husks were weighed according to the quantity required for each treatment. Five coco peats and burnt paddy husks mixtures were used as treatments in this study. These treatments were as follows: T1 = 100% coco peat; T2 = 100% burnt paddy husks; T3 = 70% coco peat and 30% burnt paddy husks; T4 = 30% coco peat and 70% burnt paddy husks; and T5 = 50% coco peat and 50% burnt paddy husks. Each mixture was thoroughly mixed in a 10-litre pail before filling into 16 cm x 16 cm black polyethene bags. The seed rhizomes were sown into the media according to the treatments. Each polyethene bag was placed randomly on four irrigation lines under the side-netted rain shelter and individually irrigated with nutrient solution via a dripper on the surface of the medium.

## 2.4 Irrigation set-up

The irrigation system, built in the side-netted rain shelter, consisted of a 1,500-litre tank, 1.5 Hp water pump, water filter, pressure meter and four lateral lines (28 m each) looped to each other. Each lateral line was equipped with 100 drippers placed into 100 polyethylene bags, side by side. The distance between each line was 1.5 m and between each dripper point in the lateral line was 0.3 m. A valve was attached to an inlet to control the amount of the irrigated solution pumped in. A small valve was also attached to each lateral line to maintain the flow through the drip line. The nutrient solutions were supplied through 0.3 m micro tubes and arrow drippers.

## 2.5 Nutrient concentrations and irrigation frequencies

The fertiliser was formulated by MARDI based on the nutrient requirements of the plant rhizomes. All the fertiliser components were water soluble. The fertiliser stocks were prepared according to Yaseer Suhaimi et al. (2011). The macro and micro nutrients were prepared separately as A and B stock solutions respectively, at 100x dilution. Solution A contained calcium nitrate and iron, while solution B contained all other components. All components were added one by one to ensure that they dissolved entirely in the water. In preparing stock A solution, calcium nitrate was added into the container containing tap water (pH 5.5 – 6.5) and stirred until it dissolved, then the solution was poured into a 100-litre vessel. Iron powder was added into another container that contained tap water, stirred until it dissolved completely, and then added into the vessel. The same procedure was applied in preparing stock B solution.

The irrigation solutions were prepared in a 1,500-litre tank. Stock A and stock B were added into the tank at 1:1 ratio until the needed electricity conductivity (EC) was achieved. The EC of the fertigation solution was between 1800 µS and 2400 µS. The irrigation scheduling was automatically implemented by a digital timer, two times per day in the first 3 months (0800 h and 1600 h), three times per day in the $4^{th}$ – $7^{th}$ months (0800 h, 1000 h and 1600 h), and once per day in the last month (0800 h). The irrigation duration was 3 min and an identical amount of fertiliser solution was applied to all polyethene bags. The daily irrigation volumes per plant were 500 ml in the first 3 months, 750 ml in the $4^{th}$ – $7^{th}$ months, and 250 ml in the last month. Routine horticultural practices for pest, disease and weed control were followed. Insecticide (Malathion) and fungicide (Benlate) were applied once every 2 weeks.

## 2.6 Parameters measurements

The growth of the black ginger plants was measured monthly by measuring the height and weight of leaves/shoots and rhizomes. The black ginger plants were randomly selected, and the rhizomes were harvested after eight months of sowing to determine their growth, yield, and bioactive compound. The weight was measured immediately after harvest to prevent desiccation and water loss from the rhizomes.

## 2.7 Air-filled porosity (AFP) and container moisture capacity (CMC)

The container moisture capacity (CMC) is the amount of water present after the medium has been saturated and allowed to drain. The CMC of the five different media mixtures were taken at two different time intervals and calculated using the formula: (saturated mass – dry mass)/dry volume. The measurement was taken one month after planting by weighing the container at 1 h and 5 h after watering. Air-filled porosity (AFP) or air capacity can be defined as the proportion of the volume that contains air after it has been saturated with water and allowed to drain. The AFP measurement was done according to Bunt (1988).

## 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. Extraction was repeated three times under identical conditions. The filtrates were combined and brought to complete dryness using a rotary evaporator to obtain the crude extracts. The crude extracts were stored at 4°C till the following 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 amount of 4,5,7-trimethoxyflavone in the extracts was calculated using the regression equation of its peak area to peak area of known concentration of the standard from the calibration curve.

## 3.0 Statistical analysis

Data obtained were subjected to statistical analysis using analysis of variance (ANOVA) procedures to test the significant effect of all the variables investigated using SAS version 9.1. Means were separated using the Duncan Multiple Range Test (DMRT) as the test of significance at $p \leq 0.05$.

## 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 1 h and 5 h of irrigation (Table 1). Meanwhile, there were no significant differences in the AFP value between 50% coco peat and 50% burnt paddy husks mixture and 70% coco peat and 30% burnt paddy husks mixture. 100% burnt paddy husks (initial: 6.7%/final: 9.4%) had the second lowest initial and final porosity at both times after irrigation, followed by 30% coco peat and 70% burnt paddy husks mixture (initial: 5.8%/final: 7.8%). The AFP value from 100% coco peat and mixture with higher coco peat (up to 70%) increased compared to 100% burnt paddy husks and mixture with higher burnt paddy husks (up to 70%). Mixtures with a high content of burnt paddy husks had lower AFP values due to their compaction and high water retention properties. The air volume increased when coco peat was mixed into burnt paddy husks. The addition of coco peat increased the air capacity and decreased the water contents of the mixtures. The availability of air in the substrate is an important factor affecting the success of growing plants in containers [25]. The container moisture capacity (CMC) measures the water availability or content in the growth substrate. The CMC values decreased 5 h after irrigation (Table 1). The highest initial and final CMC values were obtained from the mixture of 30% coco peat and 70% burnt paddy husks, followed by 100% burnt paddy husks, mixture of 50% coco peat and 50% burnt paddy husks, mixture of 70% coco peat and 30% burnt paddy husks and the lowest CMC was observed in the 100% coco peat substrates. The differences in CMC values between 100% burnt paddy husks and 100% coco peat were 26.7% and 22.2% respectively in both times after irrigation. These results showed that adding burnt paddy husk into coco peat increased the moisture content while lowering the AFP of the substrates. The air retention and moisture in the substrate play important roles for thriving plant growth in containers [25].



Table 1: Physical properties of growth substrates at 2 different times after irrigation

| Treatment | Air-filled | Container moisture |
|---|---|---|

| | porosity (%) | | capacity (%) | |
|---|---|---|---|---|
| | 1 h | 5 h | 1 h | 5 h |
| 100% CP | 9.8[a] | 14.0[a] | 41.5[e] | 36.8[e] |
| 100% BPH | 6.7[c] | 9.4[c] | 68.2[b] | 59.0[b] |
| 70% CP + 30% BPH | 8.4[b] | 10.3[b] | 51.0[d] | 47.3[d] |
| 30% CP + 70% BPH | 5.8[d] | 7.8[d] | 71.4[a] | 67.6[a] |
| 50% CP + 50% BPH | 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; BPH = Burnt paddy husks



### 3.2 Effects on plant growth

There were significant differences in vegetative fresh weight between treatments at *p* ≤0.05 (Table 2). The highest vegetative fresh weight was produced by black ginger cultivated in 50% coco peat and 50% burnt paddy husks 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% burnt paddy husks mixtures that can retain suitable moisture in the substrates compared to the other treatments. Coco peat as substrate has a higher porosity compared to burnt paddy husk, which has low porosity. Porosity characteristics allow the substrate to retain moisture and create air-filled space in the substrates. 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 burnt paddy husks 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% burnt paddy husks 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 burnt paddy husks 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].

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% BPH | $60^{a}$ | $582^{c}$ | $22^{b}$ | $48^{c}$ | $0.14^{a}$ | $228^{d}$ | $0.39^{d}$ |
| 70% CP + 30% BPH | $56^{a}$ | $620^{b}$ | $18^{c}$ | $52^{b}$ | $0.12^{a}$ | $418^{b}$ | $0.68^{b}$ |
| 30% CP + 70% BPH | $59^{a}$ | $548^{d}$ | $25^{a}$ | $58^{a}$ | $0.14^{a}$ | $298^{c}$ | $0.55^{c}$ |
| 50% CP + 50% BPH | $58^{a}$ | $678^{a}$ | $22^{b}$ | $50^{b}$ | $0.13^{a}$ | $582^{a}$ | $0.86^{a}$ |

Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$

CP = Coco peat; BPH = Burnt paddy husks

### 3.3 Effects on rhizome yield

For commercial purposes, black ginger rhizomes are 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 were darker purple than the mother seed piece. The rhizomes also produced a rancid odour. There were significant differences in rhizome yield between treatments after eight months of cultivation (Table 2). The highest average fresh rhizome yield was obtained from plants cultivated in 50% coco peat and 50% burnt paddy husks, followed by mixtures of 70% coco peat and 30% burnt paddy husks, 30% coco peat and 70% burnt paddy husks, 100% burnt paddy husks, and last, 100% coco peat. These results showed that black ginger cultivated in the equivalent of the amount of coco peat media and burnt paddy husks 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% burnt paddy husks 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 burnt paddy husks 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% burnt paddy husks 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% burnt paddy husk mixtures are less acidic with a pH suitable to facilitate ginger growth, allowing the plant roots to absorb nutrients efficiently.

Media with high content of coco peat gave lower rhizome yield throughout the cultivation period; a mixture of 100% coco peat exhibited the lowest rhizome yield. These results were similar to the study conducted by Wan Zaliha (2018), who found that the yield of black ginger rhizome decreased significantly when grown using 100% coco peat. A Previous study by Hayden et al. (2004) found that the growth of rhizomes depends on the medium type. The growing medium acts as a heat insulator and provides heat that enhances the growth of rhizomes. The overall biomass of black ginger plants can be divided into aboveground biomass consisting of leaves and stems (shoots) and underground biomass consisting of rhizomes and roots. This study showed significant differences between treatments in rhizomes to shoot ratio. The ratio of underground biomass to aboveground biomass was highest in plants cultivated in 50% coco peat and 50% burnt paddy husks mixtures with a ratio of 0.86 (Table 2). There was higher underground biomass compared to aboveground biomass in all treatments. The high ratio of underground biomass to aboveground biomass reflects that the roots could supply the top of the plant with water, nutrients, stored carbohydrates and certain growth regulators [36].

### 3.4 Bioactive compound

Figure 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% burnt paddy husk sample gave the lowest values for 4,5,7-trimethoxyflavone. The various combinations of coco peat and burnt paddy husk 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.

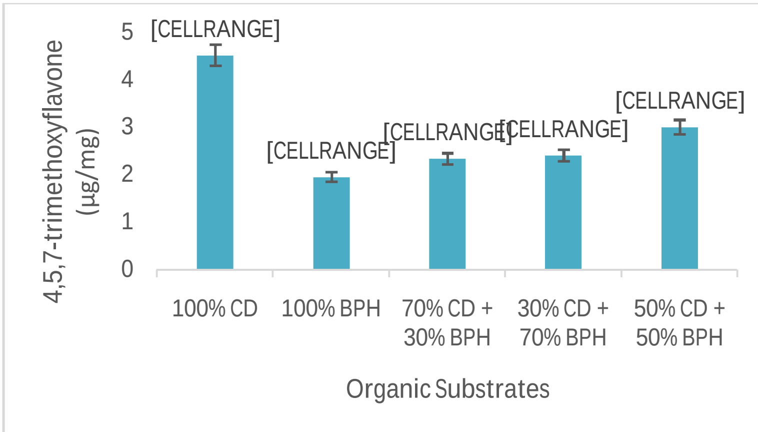


Figure 1: Effect of substrates on 4,5,7-trimethoxyflavone accumulation in the black ginger rhizomes after eight months of cultivation

In contrast, the rhizomes grown in 100% burnt paddy husk showed the lowest 4,5,7-trimethoxyflavone content at 1.959 µg/mg. This suggests that burnt paddy husk, 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 burnt paddy husk resulted in intermediate levels of 4,5,7-trimethoxyflavone, with the combination of 50% coco peat + 50% burnt paddy husk 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 burnt paddy husks significantly alters the characteristics of substrates 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% burnt paddy husks 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% burnt paddy husk mixtures are the best substrates 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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