

ARTICLE

Utilization of Spent Mushroom Substrate and Local Organic Wastes as Sustainable Growth Media for Enhanced Cocoa Seedling Development

Karupakorn Laeid-on ^{*} , Arunrussamee Sangsila, Worrawat Promden, Tepporn Lomarak

Department of General Science, Buriram Rajabhat University, Buriram 31000, Thailand

ABSTRACT

This study examines the effects of germination substrates incorporating spent mushroom substrate (SMS) and locally sourced organic materials on the growth and vigor of cocoa seedlings. Seven treatments were evaluated using a Completely Randomized Design (CRD), comprising combinations of coconut coir, rice husk, sugarcane bagasse, and biochar. Key findings reveal that treatments combining SMS, sugarcane bagasse, and either coconut coir or biochar (T6 and T7) achieved significantly higher survival rates and Dickson Quality Index (DQI) scores compared to conventional soil (T1). These treatments provided favorable chemical properties, including optimal pH, electrical conductivity, and nutrient content (N, P, K), which are critical for cocoa seedling development. The results suggest that using SMS with agricultural waste as an alternative germination medium can reduce cultivation costs, enhance seedling growth, and contribute to sustainable agricultural practices by repurposing organic waste. This approach not only offers cost-effective benefits for farmers but also mitigates environmental impacts associated with waste disposal.

Keywords: Spent Mushroom Substrate; Organic Materials; Seeding Cocoa

*CORRESPONDING AUTHOR:

Karupakorn Laeid-on, Department of General Science, Buriram Rajabhat University, Buriram 31000, Thailand; Email: Kluphakorn.Li@bru.ac.th

ARTICLE INFO

Received: 10 September 2024 | Revised: 10 October 2024 | Accepted: 15 October 2024 | Published Online: 9 January 2025

DOI: <https://doi.org/10.30564/jees.v7i1.7253>

CITATION

Laeid-on, K., Sangsila, A., Promden, W., et al., 2025. Utilization of Spent Mushroom Substrate and Local Organic Wastes as Sustainable Growth Media for Enhanced Cocoa Seedling Development. *Journal of Environmental & Earth Sciences*. 7(1): 540–549.

DOI: <https://doi.org/10.30564/jees.v7i1.7253>

COPYRIGHT

Copyright © 2025 by the author(s). Published by Bilingual Publishing Group. This is an open access article under the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) License (<https://creativecommons.org/licenses/by-nc/4.0/>).

1. Introduction

The cocoa plant, scientifically named *Theobroma cacao* L., belongs to the Sterculiaceae family. This small perennial tree thrives in shade and high humidity conditions, predominantly cultivated in South America and West Africa^[1]. Research and development efforts focus on breeding, disease prevention, and enhancing yield. The lack of shade in monoculture plantations can lead to excessive sunlight exposure, causing leaf burn and adversely affecting flower and fruit growth, ultimately reducing yield^[2]. Cocoa is nutritionally valuable, containing carbohydrates and fats^[1]. Its extract comprises essential nutrients and phytochemicals, including proteins, carbohydrates, fats, tannins, flavonoids, terpenoids, and alkaloids^[3]. Cocoa protein contains vital amino acids such as glutamate, aspartate, valine, and alanine^[3] making it sought after in the food processing industry for the production of confections, chocolate, beverages, cosmetics, pharmaceuticals, and cocoa-enhanced foods. The price of cocoa is trending upwards^[4] reflecting its growing value in various industries^[5].

Thailand initiated its interest in cultivating cocoa in 1913 by experimenting with hybrid varieties imported from Malaysia. By 1979, a collection of 34 strains from various countries, including Malaysia, the United States, and the United Kingdom, was planted at the Chumphon Horticultural Research Center. This led to the development a standardized new breed named “Chumphon 1” hybrid cocoa, which consistently produces dry cocoa beans that meet international standards. Concurrently, technological advancements in management practices were developed to enhance the quality and quantity of the yield. These advancements have made it appealing to propagate cocoa seedlings to increase their potential further and improve quality yields through technological innovations in management practices, including pruning, harvesting, and processing. Moreover, encouraging farmers to cultivate in various areas has helped increase their income^[6]. However, in cocoa cultivation, challenges related to the quality of planting soil have been identified. Most farmers primarily use garden soil as the planting medium, which presents problems such as low germination rates and survival rates of plants. Some farmers have resorted to using expensive germination materials, such as peat moss, which has become increasingly popular with the rising import of foreign germination substances. Moreover, others have utilized commercially

available pre-mixed soils as a cheaper alternative for seedling cultivation, though these tend to be of lower quality. While agricultural waste materials are readily available, inexpensive, and of good quality in Thailand, their development into suitable substrates for seedling cultivation could significantly reduce production costs^[7]. Examples include a spent mushroom substrate, sugarcane bagasse, coconut coir, and rice husk ash, which are characterized by their lightweight, excellent moisture retention, good aeration, and flexibility without compaction. Rice husk ash exhibits superior water-holding capacity and allows for effective drainage and aeration^[8]. Utilizing spent mushroom substrate in cultivation helps mitigate environmental issues and improves soil structure^[9]. Other organic waste materials, such as rice straw, wood bark, and corn stalks, can serve as organic substrates, providing essential nutrients for growth and offering suitable chemical and physical properties for plant development and productivity^[10]. Thailand currently has a substantial amount of agricultural waste materials. Utilizing these leftovers constructively could significantly mitigate environmental issues. Thus, this research aims to address such challenges by focusing on the effects of using local organic materials as seedling substrates, specifically tailored for the growth of cocoa. The study seeks to develop suitable cultivation mediums from these materials, offering a strategy to enable farmers to produce their seedling substrates. This approach not only promotes self-sufficiency among growers but also contributes to reducing production costs.

2. Research Methodology

2.1. Composting of the Cultivation Substrate

In this step, one part of the spent mushroom substrate is layered to a maximum height of 20 centimeters with a base dimension of 1.20 meters by 1.20 meters, ensuring no compression. This is topped with a layer of wood chips, also one part, and the process is repeated for a total of 10 layers. A mixture of one liter of molasses and a microbial activator (PD1, Department of Land Development, Ministry of Agriculture and Cooperatives, Thailand) dissolved in water is then evenly applied to moisten each layer. The heap is constructed into a square shape with a height of 1 meter. The significance of creating thin layers, up to 10 in total, is to allow the microorganisms present in the spent mushroom substrate to utilize both the carbon from the plant debris

and the nitrogen within the substrate for growth and cell formation, thereby accelerating the decomposition of raw materials. The compost is left to ferment for two months, maintaining the moisture content of the compost pile at an optimal level (approximately 60–70%) by watering once daily. After the first 7 days, a solution of beneficial microbes in 20 liters of clean water is sprinkled over the compost pile. The pile is then turned every 15 days to ensure even moisture distribution throughout the composting period.

2.2. Preparation of Cultivation Medium

The composted spent mushroom substrate was mixed according to the ratios specified for each experimental treatment, planned to use a Completely Randomized Design (CRD) methodology. There were seven treatments, each replicated three times with 20 bags per replicate, each bag containing a single “Chumphon 1” cocoa seed. The experimental treatments were as follows:

Treatment 1 (T1): Soil (control group) at a 100% ratio (S)

Treatment 2 (T2): Coconut coir to black rice husk at a 1:1 ratio (CC : HB)

Treatment 3 (T3): Spent mushroom substrate to filter cake at a 1:1 ratio (SPM : FC)

Treatment 4 (T4): Spent mushroom substrate to raw rice husk at a 1:1 ratio (SPM : HR)

Treatment 5 (T5): Spent mushroom substrate, filter cake, and black rice husk at a 1:1:1 ratio (SPM : FC : HB)

Treatment 6 (T6): Spent mushroom substrate, filter cake, and coconut coir at a 1:1:1 ratio (SPM : FC : CC)

Treatment 7 (T7): Spent mushroom substrate, filter cake, and biochar at a 1:1:1 ratio (SPM : FC : BIO)

2.3. Seedling Cultivation

2.3.1. Seed Selection

Seeds were submerged in water to separate the viable ones, which are those that sank, indicating their completeness. The selected seeds were then cleaned and soaked in water for 24 hours to soften the outer shell and allow water to permeate the interior of the seed (**Figure 1a**).

2.3.2. Preparation of the Germination Medium

Finely ground coconut coir was moistened and used to incubate the selected seeds to initiate germination before their transfer to experimental cultivation bags. This process utilized ‘Chumphon 1’ cocoa seeds, with the experimental setup comprising three replicates, each containing 20 bags, and each bag containing one seed, totaling 420 bags (**Figure 1b**).



(a)



(b)

Figure 1. Preparation and Growth of Cocoa. (a) Cocoa seeds germinated in coconut coir; (b) Planting of cocoa seeds in bags, one seed per bag.

2.4. Data Collection and Analysis

Physical and chemical properties, including pH (1:1 soil-to-water ratio), electrical conductivity (EC), and levels of nitrogen (N), phosphorus (P), and potassium (K), were measured on day 45 of the experiment. A random selection of 15 cocoa seedlings per treatment was divided into three

replicates of five plants. Total nitrogen was determined using the semi-micro Kjeldahl method involving acid digestion, distillation, and titration^[11]. Phosphorus was extracted with the Bray II method and analyzed colorimetrically^[12], while potassium was extracted with 1N ammonium acetate (pH 7.0) and measured via flame photometry^[13].

Data on seedling growth and germination will be col-

lected, including survival rate, seedling vigor, dry weight of the shoot, dry weight of the root, total dry weight (shoot and root), fresh weight of the shoot, fresh weight of the root, total fresh weight (shoot and root), root length, stem diameter, and plant height. The survival rate will be calculated using the following formula^[14]:

$$\text{Survivalrate} = \left(\frac{\text{Totalnumberofsurvivingseedlings/}}{\text{Totalnumberofseeds}} \right) \times 100 \quad (1)$$

The measurement of seedling quality, indicated by the Dickson Quality Index (DQI), follows the method proposed by^[14] based on the formula:

$$\text{DQI} = \frac{\text{TDM (g)}}{\frac{\text{SH(cm)}}{\text{SBD(cm)}} + \frac{\text{SDM(g)}}{\text{RDM(g)}}} \quad (2)$$

DQI represents the Dickson Quality Index, TDM represents total dry matter, SH represents stem height, SBD represents stem base diameter, SDM represents the dry weight of the stem, and RDM represents the dry weight of the root.

2.5. Statistical Data Analysis

Statistical variance was analyzed in accordance with

the Completely Randomized Design (CRD) experimental layout. Mean comparisons were conducted using the Duncan's Multiple Range Test (DMRT) at a confidence level with 95%.

3. Results

3.1. Chemical Properties of the Cultivation Substrate

The study revealed that the pH levels of the cultivation substrates ranged from 6.50 to 7.60, and electrical conductivity (EC) values were between 0.003 and 0.30 ds/m. Total nitrogen content varied from 0.02 to 1.84%, available phosphorus ranged from 2.10 to 28.60 mg/kg, and exchangeable potassium levels were between 45.43 and 146.50 mg/kg (**Table 1**). Treatment 7 (SMS, sugarcane bagasse, and biochar) displayed the highest nitrogen (1.84%) and potassium (146.50 mg/kg), while Treatment 3 (SMS and filter cake) had the highest phosphorus content (28.60 mg/kg). These values provide insights into the nutrient composition and suitability of substrates for cocoa cultivation.

Table 1. Chemical properties of the cultivation substrate.

	pH	EC (Ds/M)	Total N (%)	Available P (Mg/kg)	Exchangeable K (Mg/Kg)
(T1) Soil (control)	7.60	0.003	0.03	2.10	45.43
(T2) CC: HB (1:1)	6.80	0.18	0.02	20.50	95.50
(T3) SPM : FC (1:1)	7.40	0.20	0.15	28.60	130.50
(T4) SPM : HR (1:1)	6.50	0.26	0.45	22.30	95.60
(T5) SPM : FC : HB(1:1:1)	6.60	0.21	1.65	23.50	135.50
(T6) SPM : FC : CC (1:1:1)	6.80	0.28	1.68	21.50	134.60
(T7) SPM : FC : BIO (1:1:1)	6.60	0.30	1.84	19.35	146.50

3.2. Survival Rate of Cocoa

The study measured the survival rate of cocoa seedlings under various substrate treatments. The highest survival rates, at 93.33%, were observed in both Treatment 6 (SMS, sugarcane bagasse, and coconut coir) and Treatment 7 (SMS, sugarcane bagasse, and biochar), both mixed at a ratio of 1:1:1. In contrast, Treatment 4, which used SMS and raw rice husk at a 1:1 ratio, had the lowest survival rate of 51.67%, as detailed in **Table 2**.

3.3. Cocoa Plant Height

The growth of cocoa plant height was measured at 15,

30, and 45 days after planting across various substrate treatments. At 15 days, the tallest plants, averaging 10.31 cm, were observed in Treatment 6 (SMS, sugarcane bagasse, and coconut coir in a 1:1:1 ratio). Conversely, the shortest plants, with an average height of 4.61 cm, were in Treatment 4 (SMS and raw rice husk in a 1:1 ratio).

At 30 days, cocoa plants in Treatment 2 (coconut coir and black rice husk in a 1:1 ratio) reached the highest average height of 22.86 cm, while Treatment 4 plants remained the shortest at 16.97 cm. By 45 days, Treatment 2 continued to show the tallest plants, with an average height of 24.44 cm, whereas plants in the control treatment (soil) were the shortest, averaging 18.09 cm (**Table 3**).

Table 2. Survival rate of cocoa.

Treatment	Total Number of Seeds	Total Number of Surviving Seedlings	Survival Rate (%)
(T1) Soil (control)	60	40	66.67
(T2) CC : HB (1:1)	60	42	70.00
(T3) SPM : FC (1:1)	60	46	76.67
(T4) SPM : HR (1:1)	60	31	51.67
(T5) SPM : FC : HB (1:1:1)	60	44	73.33
(T6) SPM : FC : CC (1:1:1)	60	56	93.33
(T7) SPM : FC : BIO (1:1:1)	60	56	93.33

Table 3. Height of Cocoa Plants at 15, 30, and 45 Days after Planting.

Treatment	Height (cm)		
	15 day	30 day	45 day
(T1) Soil (control)	8.17bc	17.38b	18.09c
(T2) CC : HB (1:1)	8.00bc	22.86a	24.44a
(T3) SPM : FC (1:1)	8.07bc	19.88ab	21.44b
(T4) SPM : HR (1:1)	4.61d	16.97b	21.04b
(T5) SPM : FC : HB (1:1:1)	7.25c	18.49b	20.91b
(T6) SPM : FC : CC (1:1:1)	10.31a	20.57ab	20.77b
(T7) SPM : FC : BIO (1:1:1)	9.81ab	20.12ab	21.14b
CV%	12.94	10.69	5.27

Different letters (a, b, c) in the same column indicate statistically significant differences between means according to Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

3.3.1. Morphological Responses of Cocoa Seedlings to Various Cultivation Substrates

The study examined plant height, root length, and stem diameter across different substrate treatments. The tallest cocoa plants, averaging 30.73 cm, were observed in Treatment 2 (coconut coir and black rice husk at a 1:1 ratio). In contrast, the shortest plants were in Treatment 1 (control group with soil at 100%), with an average height of 21.56 cm. Root length varied significantly among treatments, with

Treatment 6 (spent mushroom substrate, sugarcane bagasse, and coconut coir at a 1:1:1 ratio) yielding the longest roots, averaging 19.30 cm, while Treatment 1 showed the shortest roots at 13.73 cm.

The largest stem diameter was recorded in Treatments 2 and 5, where substrates included black rice husk and spent mushroom substrate, respectively, both at a 1:1:1 ratio, with an average diameter of 0.41 cm. Conversely, the smallest stem diameter was found in Treatment 1 (control soil), with an average of 0.32 cm (Table 4).

Table 4. Comparative Growth Parameters of Cocoa Seedlings under Different Treatment Conditions.

Treatment	Height (cm)	Root Length (cm)	Stem Diameter (cm)
(T1) Soil (control)	21.56c	13.73c	0.32c
(T2) CC : HB (1:1)	30.73a	15.16bc	0.41a
(T3) SPM : FC (1:1)	27.26b	17.33ab	0.40ab
(T4) SPM : HR (1:1)	26.56b	17.90ab	0.38b
(T5) SPM : FC : HB (1:1:1)	27.56b	17.13ab	0.41a
(T6) SPM : FC : CC (1:1:1)	25.53b	19.30a	0.39ab
(T7) SPM : FC : BIO (1:1:1)	25.73b	16.83ab	0.39ab
CV%	5.57	9.39	0.33

Different letters (a, b, c) in the same column indicate statistically significant differences between means according to Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

3.4. Fresh and Dry Weight of Cocoa Plant Shoots and Roots

The study measured the fresh and dry weights of cocoa plant shoots and roots under different treatments. The highest total fresh weight of stems and roots was recorded in

Treatment 2 (coconut coir and black rice husk at a 1:1 ratio), averaging 3.73 grams. In contrast, the control treatment with soil exhibited the lowest total fresh weight, averaging 2.67 grams. For the dry weight of shoots and roots, Treatments 5 and 7 (SMS, sugarcane bagasse, and black rice husk at a

1:1:1 ratio, and SMS, sugarcane bagasse, and biochar at a 1:1:1 ratio, respectively) had the highest dry weights, averaging 0.79 grams. The control treatment with soil recorded the lowest dry weight, with an average of 0.49 grams (Table 5).

Table 5. Fresh and Dry Weights of Cocoa Plant Shoots and Roots under Different Treatments.

Treatment	Fresh Weight			Dry Weight		
	Stem (g)	Root (g)	Stem and Root (g)	Stem (g)	Root (g)	Stem and Root (g)
(T1) Soil (control)	2.43b	0.47ab	2.67b	0.42b	0.07c	0.49b
(T2) CC: HB (1:1)	3.23a	0.49a	3.73a	0.66a	0.09b	0.76a
(T3) SPM : FC (1:1)	2.54b	0.40bc	2.94b	0.65a	0.11ab	0.77a
(T4) SPM : HR (1:1)	2.40b	0.31d	2.71b	0.62a	0.11ab	0.74a
(T5) SPM:FC:HB(1:1:1)	2.53b	0.29d	2.82b	0.69a	0.10ab	0.79a
(T6) SPM:FC:CC(1:1:1)	2.50b	0.38c	2.89b	0.66a	0.12a	0.78a
(T7) SPM:FC:BIO(1:1:1)	2.34b	0.35cd	2.70b	0.66a	0.12a	0.79a
CV%	7.68	9.03	7.18	7.21	0	6.12

Different letters (a, b, c) in the same column indicate statistically significant differences between means according to Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

4. Dickson Quality Index

The study measured the Dickson Quality Index (DQI) of cocoa seedlings under different substrate treatments to assess seedling quality. The highest DQI was observed in Treatment 7, which combined spent mushroom substrate, sugarcane bagasse, and biochar in a 1:1:1 ratio, with an average index of 0.066. Conversely, the lowest DQI was recorded in the control treatment using soil, which had an average value of 0.038. Table 6 provides the DQI values for all treatments.

Table 6. Dickson Quality Index of Cocoa Seedlings Under Different Treatments.

Treatment	Dickson Quality Index
(T1) Soil (control)	0.038
(T2) CC : HB (1:1)	0.051
(T3) SPM : FC (1:1)	0.061
(T4) SPM : HR (1:1)	0.059
(T5) SPM : FC : HB (1:1:1)	0.058
(T6) SPM : FC : CC (1:1:1)	0.065
(T7) SPM : FC : BIO (1:1:1)	0.066

5. Discussion

5.1. The Role of Spent Mushroom Substrate and Biochar in Cocoa Cultivation

Utilizing spent mushroom substrate as a component in planting materials presents a substrate with appropriate chemical and physical properties^[15]. Not only does it help in enhancing yield, but it also demonstrates an increase in nitrogen-fixing bacteria in the soil. It can be used for cultivating greenhouse plants, field crops, or in conjunction with

manure for planting various crops^[16].

Spent mushroom substrate can be utilized in the production of various nursery environments for plants such as shrubs and for the germination of grass seeds, all of which result in favorable yields. It can be mixed with locally available organic waste materials, including coconut coir, black rice husk, raw rice husk, sugarcane bagasse, and biochar, to evaluate its efficacy in supporting the growth of cocoa plants. Prior to testing, these materials are blended in various ratios and analyzed for their chemical composition. The majority of these planting materials exhibit a pH range of 6.50 to 7.60, while the optimal pH for cocoa growth typically falls between 5.5 and 7.0.

This indicates that the cultivation materials are mildly acidic, with the pH values for treatments 2, 4, 5, 6, and 7 being 6.80, 6.50, 6.60, 6.80, and 6.60, respectively, which are suitable for cocoa cultivation. The electrical conductivity (EC) of the cultivation materials ranged from 0.003 to 0.300 ds/m. Generally, substrates appropriate for cultivation should have an EC value with no exceeding 10 ds/m, assessing the concentration of soluble salts, including ions such as Na^+ , K^+ , Ca^{2+} , Mg^{2+} , and NH_4^+ . Substrates with high salinity levels are deemed unsuitable for plant growth.

The total nitrogen content in the cultivation substrates ranged from 0.02 to 1.84%, with treatments 5, 6, and 7 showing values of 1.65%, 1.68%, and 1.84%, respectively. Substrates suitable for plant cultivation typically contain no less than 1.0% by weight of nitrogen. Forms of nitrogen beneficial to plants include ammonia (NH_4^+) and nitrate (NO_3^-). Ammonia tends to be well adsorbed by the soil surface or

in media under neutral or increasingly alkaline conditions, while nitrates are readily available to plants in soil that is neutral to acidic. The available phosphorus content ranged from 2.10 to 28.60 mg/kg.

The cultivation substrate of treatment 3 exhibited the highest available phosphorus content, measuring 28.60 mg/kg, while the control treatment recorded the lowest value at 2.10 mg/kg. Phosphorus is a critical nutrient that is often in short supply both in natural ecosystems and agricultural systems globally, particularly in Asia. The beneficial form of phosphorus available to plants in solution typically represents a very low percentage with no exceeding 0.01% of the total phosphorus content in the soil. The exchangeable potassium content ranged from 45.43 to 146.50 mg/kg, with treatment 7 displaying the highest value at 146.50 mg/kg, and the lowest being in treatment 1 (control) at 45.43 mg/kg. Potassium is an essential nutrient for plants, second only to nitrogen and phosphorus. This is in line with research found that spent mushroom substrate contains chemical compositions of 1.51% nitrogen, 3.77% phosphorus, and 0.61% potassium. Additionally, the electrical conductivity was recorded at 6.22 m/h, with a water-holding capacity of 95.03%, pH values ranging between 7.28 and 7.75, and chloride playing a significant role in conductivity^[9].

5.2. Biochar's Contribution to Improved Cocoa Seedling Viability and Substrate Optimization

The high survival rates observed in Treatments 6 and 7, which incorporate biochar, indicate the effectiveness of this substrate in supporting cocoa seedling viability. Biochar, a soil amendment produced through the pyrolysis of biomass in an oxygen-limited environment, enhances the substrate's properties, contributing to improved plant health^[17]. Biochar's high porosity, extensive surface area, and predominantly negative surface charge enable it to adsorb and slowly release nutrients, which increases soil fertility and nutrient uptake for plants. Additionally, biochar increases pH, total nitrogen (N), available phosphorus (P), and exchangeable potassium (K), enhancing the suitability of the cultivation substrate for cocoa growth. These attributes likely contributed to the enhanced survival rates in Treatment 7. Conversely, the low survival rate in Treatment 4 (SMS

and raw rice husk) suggests that raw rice husk may not provide sufficient nutrients or favorable conditions for cocoa seedlings compared to other substrates tested. The contrasting results between Treatment 4 and the biochar-inclusive treatments underscore biochar's potential role in creating an optimal growth environment for cocoa seedlings.

5.3. Evaluating the Role of Biochar and Coconut Coir in Enhancing Cocoa Seedling Growth

The findings show that Treatments 6 and 7, which involved substrates with SMS, sugarcane bagasse, and either coconut coir or biochar, supported superior cocoa plant growth. The elevated organic matter content in these substrates likely enhanced nutrient availability and absorption, contributing to taller plants compared to other treatments. The high organic content in Treatments 6 and 7 supports the root systems, allowing them to access nutrients more efficiently, which leads to improved growth and vigor. Additionally, substrates with biochar in Treatment 7 and coconut coir in Treatment 6 provide favorable conditions for water retention and aeration, essential factors for promoting strong root development and overall plant health^[18].

On the other hand, the relatively poor performance of Treatment 4, which used raw rice husk, suggests that this substrate might lack the necessary nutrient profile or physical properties required to optimize cocoa seedling growth, as evidenced by the lower average plant height across the observed periods. The results underscore the importance of substrate composition, with biochar and coconut coir offering distinct benefits that contribute to improved growth metrics for cocoa seedlings^[19].

5.4. Enhancing Cocoa Seedling Development through Optimized Substrate Composition: Benefits of Coconut Coir, Sugarcane Bagasse, and Biochar

The observed morphological responses indicate that Treatment 2, with coconut coir and black rice husk, significantly supports plant height, likely due to its favorable aeration and drainage properties, which promote root health and overall plant vigor. The inclusion of sugarcane bagasse in Treatments 6 and 7 also contributed to improved root growth

due to its high potassium content, a by-product characteristic of sugar processing that enriches soil with nutrients^[20]. Incorporating compost with biochar offers additional advantages, such as increased pH and electrical conductivity, which improves water retention and thermal conductivity in the substrate. These properties contribute to enhanced plant growth, as evidenced by higher fresh shoot weights, increased leaf numbers, and better root density^[21]. Furthermore, the presence of bioactive compounds and enzymes in spent mushroom substrate (SMS) can suppress soilborne pathogens, enhancing soil health and providing disease resistance^[22].

The beneficial combination of SMS and biochar with sugarcane bagasse, particularly in Treatments 6 and 7, highlights the synergistic effects on cocoa seedling development. These substrates not only provide essential nutrients but also create a supportive environment that fosters robust morphological characteristics, demonstrating the potential of these alternative substrates for sustainable cocoa cultivation.

5.5. Optimizing Cocoa Seedling Growth with Coconut Coir and Biochar: Enhancing Plant Health and Supporting Environmental Sustainability

The results suggest that Treatment 2, which combines coconut coir and black rice husk, offers optimal conditions for cocoa seedling growth, as evidenced by the highest fresh weight of shoots and roots. This mixture likely enhances aeration and water retention, which are crucial for vigorous plant growth. The high dry weight observed in Treatments 5 and 7 reflects the benefits of using biochar and black rice husk, both of which provide structural support and nutrient retention in the substrate.

Biochar, specifically, has gained attention due to its role in improving soil health and mitigating environmental issues^[23]. It enhances soil structure, pH, and electrical conductivity, which in turn supports plant health by facilitating nutrient uptake. Additionally, biochar's role in reducing greenhouse gas emissions aligns with global sustainability goals, as it sequesters carbon and can help mitigate the greenhouse effect linked to human activities^[24, 25]. By integrating biochar into agricultural practices, as seen in Treatments 5 and 7, there is potential to promote sustainable agriculture that addresses both plant productivity and climate change

mitigation^[26, 27].

5.6. The Role of Biochar in Improving Substrate Quality and Supporting Sustainable Cocoa Cultivation

The findings demonstrate that Treatment 7, which includes biochar, provides superior substrate conditions, as indicated by the highest Dickson Quality Index (DQI). The high DQI in Treatment 7 suggests that the combination of spent mushroom substrate, sugarcane bagasse, and biochar contributes to balanced seedling growth, strong root-to-shoot ratios, and robust stem diameter—key indicators of seedling vigor and quality. Biochar's unique properties, including high porosity and nutrient retention, likely play a significant role in enhancing the substrate's physical structure and nutrient availability, which directly impacts the overall seedling quality^[24]. Additionally, biochar improves soil aeration and water retention, promoting optimal conditions for root development. The high DQI aligns with previous research on biochar's benefits for plant growth, particularly in enhancing nutrient uptake and soil health^[14, 25].

On the other hand, the low DQI observed in the control treatment indicates that plain soil may lack the necessary nutrients and structural qualities to support high-quality cocoa seedling development. This contrast underscores the potential benefits of incorporating organic substrates, particularly biochar, into cultivation media to improve plant health and sustainability. The findings further highlight the value of using sustainable materials like biochar, which can enhance soil properties while supporting environmental goals, such as carbon sequestration and reduced greenhouse gas emissions^[26].

6. Conclusions

Utilizing spent mushroom substrate mixed with organic materials as a cultivation medium has been shown to provide suitable properties for growth and survival rates of cocoa seedlings. The substrates in treatments 6 and 7, consisting of spent mushroom substrate, sugarcane bagasse, and coconut coir at a 1:1:1 ratio, and spent mushroom substrate, sugarcane bagasse, and biochar at a 1:1:1 ratio, respectively, demonstrated the highest survival rates, with an average of 93.33%. Furthermore, the seedling vigor index (DQI) for

these treatments was significantly higher, averaging 0.065 and 0.066, respectively. This was compared to cocoa planted in the control treatment with soil at a 100% ratio, which had an average survival rate of 66.67%, and the lowest DQI value of 0.038.

Author Contributions

K.L.-o. undertook the task of drafting the preliminary version of this manuscript. W.P. oversaw the conception and revision of the research and managed communication with the journal concerning publication. A.S. updated it and performed a data analysis. T.L. evaluated and amended the final draft. All authors have approved the final manuscript and consented to its publication.

Funding

Buriram Rajabhat University allocated a fund for publications.

Institutional Review Board Statement

The current study did not require ethical approval from the authors' institutions as it does not include human or animal subjects.

Informed Consent Statement

Not applicable.

Data Availability Statement

The authors consent to provide their study data upon request.

Acknowledgments

The research has been successfully completed with the generous support of Buriram Rajabhat University, which provided research funding for the fiscal year 2023. We are extremely grateful for the scholarly support provided by the President of Buriram Rajabhat University. The Faculty of Humanities and Social Sciences is deserving of special recognition for its exceptional efforts in organizing a project

that was designed to promote and facilitate the international dissemination of research articles.

Conflict of Interest

The authors assert that there are no conflicts of interest pertaining to this work.

References

- [1] Adeyeye, E.I., 2016. Proximate, Mineral and Antinutrient Compositions of Natural Cocoa Cake, Cocoa Liquor and Alkalized Cocoa Powders Sourced in Nigeria. *Journal of Advanced Pharmaceutical Science and Technology*. 1(3), 12–28. DOI: <https://doi.org/10.14302/issn.2328-0182>
- [2] Beg, M. S., Ahmad, S., Jan, K., et al., 2017. Status, supply chain, and processing of cocoa-A review. *Trends in food science and technology*. 66, 108–116.
- [3] Adeosun, Y.M., Oni, I.O., 2021. Effect of Drying Methods on Proximate and Antinutrients Composition of Cocoa (*Theobroma cacao*) Pod. *International Journal of Research and Innovation in Applied Science*. 6(5), 73–78.
- [4] Sajet, S., Durot, C., Sakouma, K.M., et al., 2017. Contribution of Associated Trees to Long Term Species Conservation, Carbon Storage and Sustainability: a Functional Analysis of Tree Communities in Cacao Plantations of Central Cameroon. *International Journal of Agricultural Sustainable*. 15(3), 282–302. DOI: <https://doi.org/10.1080/14735903.2017.1311764>
- [5] Calionara, W.B.M., Maheus, J.B., Leonardo, F.M., et al., 2020. Chemical Composition and Fatty Acids Profile of Chocolates Produced with Different Cocoa (*Theobroma cacao* L.) Cultivars. 40(2), 326–333. DOI: <https://doi.org/10.159/fst.43018>
- [6] Millán, L.M.R., Vargas, F.E.S., Nzihou, A., 2021. Characterization of Steam Gasification Biochars from Lignocellulosic Agrowaste towards Soil Applications. *Waste and Biomass Valorization*. 12(7), 4141–4155.
- [7] Buragohain, N., Gogoi, S., Kotoky, U., et al., 2022. Effect of Sowing Media and Variety on Seedling Root Growth and Field Performance of Early Cauliflower (*Brassica oleracea* var. botrytis). 40(12), 245–53. DOI: <https://doi.org/10.9734/AJAEES/2022/v40i121787>
- [8] Shuib, N. H., Ismail, A.I., Adinan, A., et al., 2018. Study on Biochemical Properties of Hevea brasiliensis Seeds Stored at Three Different Temperatures. *Research Journal of Seed Science*. 11(1) 1–11. DOI: <https://doi.org/10.3923/rjss.2018.1.11>
- [9] Kumbhar, S.S., Mahadik, M.A., Mohite, V. S., et al., 2014. Structural, dielectric and magnetic properties of Ni substituted zinc ferrite. *Journal of Mag-*

- netism and Magnetic Materials. 363, 114–120. DOI: <https://doi.org/10.1016/j.jmmm.2014.03.024>
- [10] Abhay, B., Shwati, P., 2021. Effect of Mulching on Horticultural Crop Production under Rainfed Condition - A Review. *International Journal of Current Microbiology and Applied Sciences*. 10(10), 693–700.
- [11] Nuansri, C., 2021. Cocoa, An Alternative Crop with a Future. *Kasikorn Newspaper*. 94(3), 61–68.
- [12] Agboola, A.A, Ayodele, O., 1987. Soil Test for Upland Rice in Southwestern Nigeria. *Fertilizer Research*. 14, 227–234.
- [13] Thomas, G.W., 1982. Exchangeable Cations. In: Page AL. (eds.). *Method of soil Analysis part 2. Agronomy 9, 2nd ed*. ASA and SSA: Madison Wisconsin, USA. pp. 595–624.
- [14] Kedar, A.R., Narute, T.K., Hasabnis, S.N., et al., 2019. Influence of Enriched Button Mushroom Spent Compost on Growth and Yield of Cabbage. *International Journal of Current Microbiology and Applied Sciences*. 8(11), 1658–1663.
- [15] Economou, C. N, Diamantopoulou, P. A, Philippousis, A.N., 2017. Valorization of Spent Oyster Mushroom Substrate and Laccase Recovery through Successive Solid State Cultivation of *Pleurotus*, *Ganoderma* and *Lentinula* Strains. *Applied Microbiology and Biotechnology*. 101(12), 5213–5222. DOI: <https://doi.org/10.1007/s00253-017-8251-3>
- [16] Becher, M., Magdalena, B., Agnieszka, G., 2021. Organic Matter Properties of Spent Button Mushroom Substrate in the Context of Soil Organic Matter Reproduction. *Agronomy*. 11(2), 204.
- [17] Agarwal, H., Kashyap, V.H., Mishra, A., et al., 2022. Biochar-based Fertilizers and Their Applications in Plant Growth Promotion and Protection. *3-Biotech*. 12(6), 136. DOI: <https://doi.org/10.1007/s13205-022-03195-2>
- [18] Shukla, N., Sahoo, D., Remya, N., 2019. Biochar from Microwave Pyrolysis of Rice Husk for Tertiary Wastewater Treatment and Soil Nourishment. *Journal of Cleaner Production*. 235, 1073–1079.
- [19] Mikhaylov, A., Moiseev, N., Aleshin, K., et al., 2020. Global Climate Change and Greenhouse Effect Entrepreneurship and Sustainability issues. *Journal of Entrepreneurship and Sustainability Issues*. 7(4), 2897–2913.
- [20] Soegianto, H., Ma'as, A., Nurudin, M., et al., 2017. The Effects of Filter Cake and Bagasse Ash to Growth and NPK Uptake by Sugarcane (*Saccharum Officinarum* L.) at Ultisols in Tulang Bawang, Lampung, Indonesia. *Ilmu Pertanian (Agricultural Science)*. 2(3), 112–118.
- [21] Kafle, A., Singh, S., Singh, M., et al., 2024. Effect of Biochar-compost Amendment on Soilless Media Properties and Cucumber Seedling Establishment. *Technology in Horticulture*. 4, e001. DOI: <https://doi.org/10.48130/tihort-0023-0029>
- [22] Wang, X., Liu, M., Zhang, C., et al., 2018. Antioxidant Activity and Protective Effects of Enzyme-Extracted *Oudemansiella radiata* Polysaccharides on Alcohol-Induced Liver Injury. *Molecules*. 23(2), 481. DOI: <https://doi.org/10.3390/molecules23020481>
- [23] Jeffery, S., Verheijen, F.G., Kammann, C., et al., 2016. Biochar effects on methane emissions from soils: a meta-analysis. *Soil Biol Biochem*. 101, 251–258. DOI: <https://doi.org/10.1016/j.soilbio.2016.07.021>
- [24] Alvarez, J.M., Pasion, C., Lal, R., et al., 2017. Vermicompost and Biochar as Substitutes of Growing Media in Ornamental-plant Production. *Journal of Applied Horticulture*. 19(3), 205–214.
- [25] Marjenah, Kiswanto, Sri Purwanti., et al., 2016. The Effect of Biochar, Cocopeat and Sawdust compost on the Growth of Two Dipterocarps Seedings. *Nusatara Bioscience*. 8(1), 39–44.
- [26] Aryal, J.P., Rahut, D.B., Sapkota, T.B., et al., 2019. Climate change Mitigation Options among Farmers in South Asia. *Environment, Development and Sustainability*. 22(4), 3267–3289.
- [27] Olorunfemi, I.E., Fasinmirin, J.T., Ojo, A.S., 2016. Modeling of Cation Exchange Capacity and Soil Water Holding Capacity from Basic Soil Properties. *Eurasian Journal of Soil Science*. 5(4), 266–274.