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Circular Economy Approach: Cultivating Grey Oyster Mushroom using Cassava (Manihot esculenta) Peel Waste from Starch Production

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Abstract. Cassava (Manihot esculenta) is an important staple crop in Vietnam and is estimated to produce about 10.7 million tons of starch annually. The starch production process generates a large amount of solid waste, namely cassava peels, which are currently disposed of directly into the environment. This practice poses a risk of generating foul odor, attracting disease-carrying organisms, and directly affecting human health. Therefore, reusing cassava peels for growing gray oyster mushrooms is the most appropriate solution. First and foremost, the cyanide content in cassava peels must be treated using the Sun-drying method. Results showed that the cyanide level decreased to the optimal level of 0 mg/kg from 34 mg/kg. After reusing cassava peels to grow gray oyster mushrooms, the results showed that the highest yield was 272 g/bag for cassava peels substrate, while the highest yield for sawdust substrate was 293 g/bag. Statistical evaluations showed that reusing cassava peels to replace sawdust substrate for growing gray oyster mushrooms also resulted in an equivalent yield. Utilizing the substrate after growing mushrooms on cassava peels to grow cabbage produced a germination rate and plant height development equivalent to those grown on commercial fertilizer substrate.

1 Introduction

Cassava (*Manihot esculenta*) is a perennial root crop, belonging to the Euphorbiaceae family. It can live for many years and is grown in over 100 countries with tropical and subtropical climates, mainly concentrated in Africa, Asia, and South America [1]. The Food and Agriculture Organization of the United Nations (FAO) ranks cassava as an important food crop in developing countries, after rice, maize, and wheat. Cassava starch is a crucial component in the diet of over a billion people in third world countries [2]. Additionally,

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cassava is an essential ingredient in animal feed in many countries around the world and is also a valuable export commodity used for producing sweeteners, confectionery, instant noodles, particleboard, packaging, biodegradable films, and pharmaceutical additives. Particularly, cassava is the main feedstock for the biofuel industry (ethanol) in some Asian countries [3]. In 2016, the global production of cassava reached over 277 million tons of fresh roots [4], representing a 63% increase from the year 2000, when only about 176 million tons of fresh cassava roots were produced.

The process of cassava starch production generates a significant amount of waste, while the wastewater is treated, the cassava pulp is often used as animal feed or substrate for mushroom cultivation. However, the cassava peel still lacks an efficient processing method and is usually directly discarded into the surrounding environment. The production process of starch generates a considerable amount of cassava pulp and peel waste [5]. Regarding the cassava peel waste, approximately 78-104 tons/day of solid waste is produced. This waste is collected in a landfill, which is often affected by tropical weather, leading to fermentation and unpleasant odor [6]. Many studies have attempted to utilize this waste as fertilizer, but it is challenging due to the high cellulose content, which makes natural decomposition difficult [7, 8]. Furthermore, since cassava roots and peels contain cyanide [9], their unprocessed application can pose risks to human health and the ecosystem. Improper disposal of cassava waste causes significant problems, including unpleasant odor, attracting disease-carrying organisms, and directly affecting human health.

Currently, the grey oyster mushroom is a popular edible fungus that holds significant value in tropical countries [10]. This food has high nutritional value, providing a considerable amount of protein, carbohydrates, vitamins, and minerals, and is also a valuable medicinal herb for maintaining and protecting health, as well as a valuable source of export revenue. Successful experiments have been conducted in replacing traditional sawdust with materials such as rice straw, grass, coconut coir, and sugarcane bagasse. Fortunately, cassava peel waste has been found to contain 42.6% carbohydrates, 1.6% protein, 12.1% ether extract, 5.0% total ash, and 22.5% crude fiber [11, 12]. Based on the remaining nutritional content of cassava peel waste, it can be seen that this material is suitable for replacing traditional sawdust.

Therefore, this study experimented with reusing cassava peel waste from a starch factory to cultivate grey oyster mushrooms. With the current trend of industries moving towards sustainable development and promoting solid waste treatment technology, the research aims to provide an effective, safe, and economically feasible method for treating Cyanide in cassava peel waste and opening up opportunities for its reuse in agriculture [13]. This is a typical case to help agricultural processing companies solve waste problems in a circular economy context. Additionally, cassava peel waste can be used as a new raw material to replace traditional sawdust, which is currently scarce and expensive. This also helps mushroom-growing areas save material costs and increase income for this type of production.

2 Materials and research methods

2.1 Research Materials

2.1.1 Co-mixed substrate materials

The cassava peelings were obtained from Hung Duy cassava starch production plant in Chau Thanh District, Tay Ninh Province, Vietnam. The mushroom cultivation additives, including urea fertilizer, corn and rice bran, were commercially purchased from Long

Khanh market in Dong Nai, Vietnam. The gray oyster mushroom spawn was obtained from Luong Xuan Yen gray oyster mushroom cultivation facility in Dong Nai, Vietnam. The 50-hole seedling tray, sweet cabbage seeds, Con co SS - Born AT 02 fertilizer, and Tribat soil were purchased from a local agricultural supply store in Dong Nai, Vietnam.

2.1.2 Method to evaluate the possibility of replacing cassava peel for sawdust for mushroom cultivation

The cassava peels were sun-dried to reduce cyanide levels [14]. The process involved spreading the samples on a crop netting until enough cassava peels were collected, then covering the netting and drying for 4-5 days under direct sunlight. Samples after processing and control samples (unprocessed) were analyzed for cyanide levels using AOAC 915.30 2012 method [15].

The steps for packaging substrate bags for mushroom cultivation include (1) selecting and processing raw materials. Cassava peels (treated for cyanide) were used as a replacement for traditional sawdust. The cassava peels were ground and sieved to remove impurities, then mixed with lime water and stirred until reaching 45-50% humidity, and left for 7-8 days to ensure 65%-70% humidity, being stirred every 2-3 days. (2) Creating substrate bags by mixing the materials from step 1 with nutrient substances such as urea, corn bran, and rice bran in the proportions listed in Table 1 [16]. A total of 30 substrate bags were made using cassava peels as the main material, and 30 substrate bags were made using sawdust according to the traditional formula as control samples.

Table 1. Nutritional composition in the substrate bag 1.3 - 1.5kg growing *Grey Oyster* mushroom.

Num	Nutritional ingredients	Control sample with sawdust (traditional recipe)	Test sample with cassava peel
2	Corn bran	600g	600g
3	Rice bran	300g	300g
4	Urea fertilizer	30g	30g
5	Lime Powder	600g	600g
6	Sawdust	70%	0
7	Cassava peel	0	70%
8	Additional water	Balanced depending on humidity around 50 - 60%.	

Next is step (3), processing the substrate bags for growing mushrooms by packing the processed substrate into 15×35 cm polypropylene bags. It is important to tightly compress the substrate to allow for fiber pull and provide sufficient nutrients for mushroom growth. Then, close the bags using a knot or twist-tie. After this process, all substrate bags are put into a sterilization pot and heated to a temperature fluctuating between 95 and 100°C for 4-5 hours.

The next step (4) is to inoculate the mushroom spawn into the substrate bags. The sterilized bags are left to cool down for 10-12 hours in a ventilated area to allow the temperature of the bags to decrease. The next step is to inoculate the grey oyster mushroom spawn into the substrate bags. The inoculation process must be carried out in a sterile environment, and proper hygiene must be observed, such as wearing a mask and gloves to avoid contamination. After inoculation, the bags are placed on racks at a cool temperature between 20-25°C for storage

During the storage process (5), the bags are kept for about 25-30 days, depending on the season and weather, to allow the mycelial threads to grow and form a homogeneous, solid structure. To promote mycelial growth, the environment must be kept clean, with minimal wind exposure, and a suitable humidity level of 75%. Lime powder is added to prevent contamination and keep the environment clean. When the mycelial threads have evenly colonized the substrate, the bags are opened at the top to allow for the formation of fruiting bodies.

The evaluation criteria and comparison of fruiting bodies between experimental treatments include (1) color, comparing the shade, brightness, or color changes to assess fruiting body quality, (2) morphology, comparing size and shape changes to evaluate the yield and efficiency of mushrooms, (3) comparing the fresh weight of the harvested mushrooms by weighing and comparing the total weight of mushrooms between the experimental and control groups to evaluate the yield of fruiting bodies, and (4) measuring the height, width, and length of the fruiting bodies to evaluate the yield of fruiting bodies.

2.2 Experimental method for reuse of substrate bags after growing Grey Oyster mushroom as fertilizer for broccoli

This experiment examines the germination ability of Brassica juncea seeds grown on a substrate made of a mixture of mushroom compost and clean soil. The control group for comparison consists of clean soil mixed with Con co SS - Born AT 02 commercial fertilizer.

Firstly, the seeds need to be treated before sowing. The seeds are soaked in warm water at 40-50°C for 8 hours. The physical properties of the mushroom compost substrate, after being mixed with clean soil, are treated similarly to the Con co SS - Born AT 02 fertilizer control group. On a 50-hole seedling tray, the first 5 rows are filled with the soil mixed with Con co SS - Born AT 02, while the next 5 rows are filled with the soil mixed with the mushroom compost from after mushroom cultivation. The experimental design is arranged alternately and repeated on five seedling trays (50 holes/tray).

After sowing one seed in each hole, the following criteria are observed and recorded after 7 days: (1) germination rate and (2) seedling height [17].

The data is entered and statistically analyzed using Excel 2021 and evaluated for differences using SPSS 20 software.

3 Results and discussion

3.1 Evaluation of cyanide removal efficiency in fresh cassava peel materials.

Three repeated samples were taken from fresh cassava peel at the factory and after processing, residual cyanide levels were evaluated. The results showed that the fresh cassava peel samples had relatively high levels of residual cyanide. This indicates that without an effective cyanide treatment solution, these materials not only cannot be reused, but may also pose a health risk to humans.

Table 2. Cyanide levels in fresh and processed cassava peel.

Repeated sample	HCN (mg/kg)	Note
1	0	Treated cassava peel
2	0	
3	0	
4	36.39	Fresh cassava peel
5	35.20	
6	37.37	

The results of the analysis presented in Table 2 show that the cyanide content, as determined by the AOAC 2012 (915.30) method, in fresh cassava peels was 36.32 mg/kg, while in the processed samples, it was 0 mg/kg. These values were compared to the cyanide content analyzed by the Center for Standardization and Quality Measurement Techniques 3 (Quatest 3), which found the content in fresh cassava peels to be 34 mg/kg and 0 mg/kg in the processed samples. Therefore, it can be concluded that the Sun-drying method can reduce the cyanide content of fresh cassava peels from 34 mg/kg to 0 mg/kg. This finding suggests that cassava peels can be safely reused for growing grey oyster mushrooms, providing the agriculture industry and the public with a new source of materials to replace traditional ones. Additionally, the reuse of cassava peels can replace other materials to reduce material costs and industrial waste from cassava starch processing.

3.2 Evaluation of the possibility of using cassava peel to grow Grey Oyster mushrooms.

The developmental stages of the mushroom spawn are described in Figure 1. Results from Figure 1A show that at this stage, the mycelium begins to pull the fibers down from the stalk, and the speed at which this occurs depends on the humidity conditions both inside and outside of the environment, as well as the nutrients present. At this point, mycelial growth is relatively stable.

Fig. 1. The developmental stages of fungal spores in embryo sacs from (A) after 2 weeks; (B) after 4 weeks; (C) after 5 weeks and (D) after 6 weeks.

After four weeks, Figure 1B shows that the mycelium has grown very well. Cases where the spawn bags cannot be pulled may be due to the humidity inside the bags being affected by the steam sterilization process, causing the mycelium fibers to be difficult to move across the surface of the material. Risks in mycelial development include mushroom knots and old knots that can cause the bags to be damaged and the mycelium to be unable to pull. This can also make it difficult to pull the fibers. Figure 1C shows the results of the spawn bags after five weeks. At this stage, the mycelium has spread to more than half of the bag. The ability of the mycelium to spread quickly or not is related to the mixing of nutrient components. In addition, preventing the appearance of mold during the early stages of inoculation is also very important. After six weeks with results like Figure 1D, the mycelium has spread throughout the bag. At this stage, the spawn bags are ready to form fruiting bodies. At this time, watering should be done 2-3 times per day and space should be created to allow the mushrooms to develop. After a period of 6-7 weeks, the spawn bags will produce the first fruiting bodies of gray oyster mushrooms, as shown in Figure 2.

Fig. 2. (A) The cassava peel substrate bag produced the first fungal fruiting bodies and (B) after the fungus was aged.

After cutting and opening the bag, the next day the gray oyster mushrooms began to appear as shown in Figure 2A. At this stage, only mist spraying at the bottom of the bag is necessary to provide the mushroom with suitable humidity of 70-85% for optimal growth. By the 4th day, the mushroom has transitioned to the mature stage as shown in Figure 2B. Harvesting should be done during the mature stage when the mushroom cap has transformed from a funnel shape to a flat shape with a thin and wide cap. If the edge of the cap is slightly curled or twisted, the mushroom is already mature. Mushrooms harvested at this stage have high nutritional quality, are less damaged (the edge of the cap does not break when harvested), and are easy to preserve (remain fresh for a long time). Avoid allowing the mushrooms to become too mature as it will deplete the nutrient source for the next harvest, reducing both the yield and quality of the mushrooms. Mushroom harvesting should be done on the 3rd day after the fruiting body is formed.

After the harvesting process, the fruiting bodies of gray oyster mushrooms are evaluated and compared for quality between the cassava and sawdust substrates (control sample). Figure 3A shows the results of the horizontal ear size of gray oyster mushrooms with a range of 25% to 75% fluctuating between 10.3-12.1 cm for cassava substrate, and 10.7-12.2 cm for sawdust substrate. Furthermore, both substrates reached the highest level of 12.8 cm. Overall, both cassava and sawdust substrates are relatively similar in this regard. The vertical ear size of gray oyster mushrooms ranges from 25% to 75%, fluctuating between 10.3-12.1 cm for cassava substrate and 9.9-11.5 cm for sawdust substrate. Additionally, the sawdust substrate reached the highest level of 12.9 cm, while cassava substrate reached a similar level of 12.5 cm. Overall, both cassava and sawdust substrates are relatively equivalent in this regard.

The results regarding the total weight of grey oyster mushrooms in Figure 3B show a fluctuation between 25% and 75%, with a range of 238-259 g for cassava peel and 238-257 g for sawdust, respectively. In addition, the highest sawdust substrate achieved was 293g, while the cassava peel substrate was nearly equivalent at 272g. Overall, both substrates of cassava peel and sawdust were quite consistent with a range of 25% - 75% fluctuation. The color of the mushroom's ear did not differ, with both having a milky white or gray color, indicating that the cassava peel did not affect the color of the grey oyster mushroom. Therefore, in this regard, the two substrates are equivalent to each other. The morphology of the ear did not change, with both having a slightly inclined cap, not straight, and a round ear. The cassava peel substrate still ensured the morphology of the grey oyster mushroom, similar to that of the sawdust substrate.

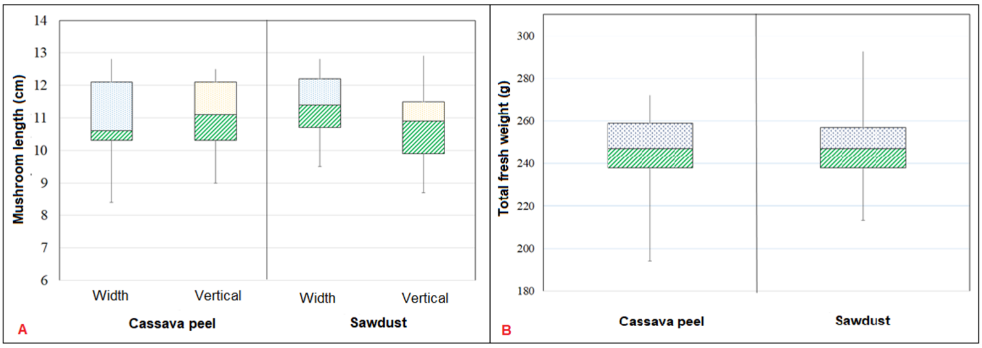

Fig. 3. (A) Ear length and (B) Fresh weight of Grey Oyster mushroom grown from cassava peel and sawdust substrate.

In general, although the yield is almost equal to that of sawdust, it also shows us the potential of cassava peel for reuse in the cultivation of grey oyster mushrooms, which also yields a high productivity.

3.3 Evaluation of Repurposing Post-Cultivation Mushroom Substrates as Organic Plant Growth Media.

The results of the evaluation of seed germination showed that for sweet cabbage plants using fertilizer from cassava residue after growing gray oyster mushrooms, there were 230 germinated plants out of a total of 250 seeds planted. For *Brassica juncea* plants using Con co SS - Born AT 02 fertilizer, there were 235 germinated plants out of a total of 250 seeds planted. Overall, the germination rate was significant and the cabbage plant ratios between the two treatments were similar. This suggests that there is great potential for using cassava residue after growing gray oyster mushrooms as fertilizer.

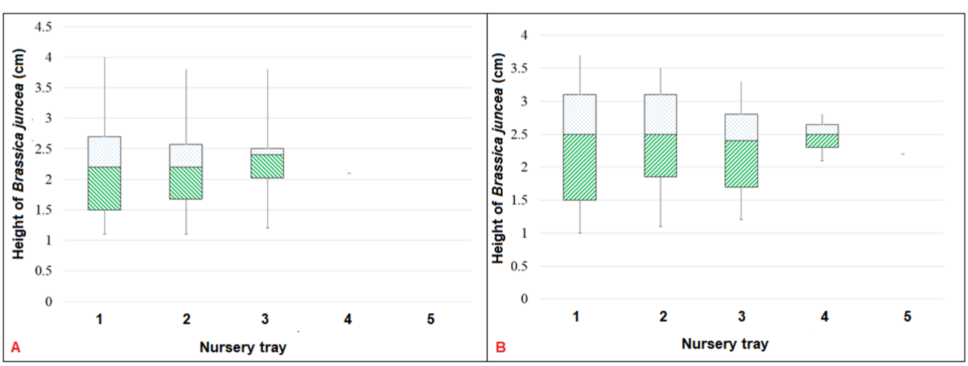

Fig. 4. The height of the Brassica juncea in the 7-day period on the substrate of (A) cassava peel and (B) commercial fertilizer used after mushroom cultivation.

Results from Figures 4A and 4B show that *Brassica juncea* plants grown on cassava residue after growing mushrooms have a minimum height of 1.1 cm and a maximum height of 4 cm. Whereas *Brassica juncea* plants grown on commercial fertilizer SS-Born AT 02 have a minimum height of 1 cm and a maximum height of 3.7 cm. The height of Brassica juncea plants in both treatments is similar with no significant difference. Therefore, after one week of evaluation, *Brassica juncea* plants have relatively stable growth in both treatments. In comparison to other studies assessing the germination of Brassica juncea, the germination rate observed in this study was found to be similar to that reported in some other studies (with an over 80% germination rate). Additionally, the plant height during the corresponding growth period showed similar results [18, 19]. This demonstrates the equivalence in supporting the germination process of the residue after mushroom cultivation and commercial fertilizer.

4. Conclusion

The composition of cassava peels contains a high amount of cellulose and a toxic substance called cyanide (HCN) at a level of 34 mg/kg. If improperly disposed of, it has the potential to cause adverse environmental effects. The Sun-drying method can completely remove the existing cyanide and meet the utilization potential. The test for the ability to substitute sawdust and reuse cassava peels as a substrate for growing oyster mushrooms showed that the highest harvested total weight of oyster mushrooms was 272 g/bag, while the sawdust substrate achieved a level of 293 g/bag. This demonstrates the potential of cassava peels to replace traditional sawdust waste to increase the value of waste materials. Additionally, the waste materials from the mushroom-growing bags also have useful value as agricultural fertilizer, with experiments on sweet cabbage showing a germination efficiency nearly equivalent to that of commercial fertilizer use. These results provide data for starch cassava enterprises to refer to when establishing a circular waste economic model for sustainable development and greater economic value. Although further testing phases are needed, the economic value of utilizing cassava peels as a substrate for growing oyster mushrooms and as a fertilizer is promising

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