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Growth of Trichoderma harzianum using starch-based household kitchen wastes

Mary Rose T. Escalante¹, Ricky B. Acanto², Marjohn Thomas N. Conlu³, Mary Grace F. Langcov², Susan V. Lirazan², Julie Ann B. Mario²

¹Negros Occidental High School, Negros Occidental, Philippines

²Carlos Hilado Memorial State University, Talisay City, Negros Occidental, Philippines ³Iloilo Science and Technology University, Miag-ao, Iloilo, Philippines Corresponding email: <u>ricky.acanto@chmsc.edu.ph</u>

ABSTRACT

Household kitchen wastes (HKW) are escalating due to rapid population growth and urbanization. To address the negative impact of throwing the HKW, the utilization and production of valuable bio-resourced potential for farmers are crucial. The study aimed to determine the growth of Trichoderma harzianum using discarded cassava, taro, sweet potato, and arrowroot peelings collected from households. Oven-dried wastes were extracted in vacuo to obtain the starch, used as alternative culture media, and compared with potato dextrose agar (PDA). The result showed that T. harzianum growth in formulated culture media had similar surface color characteristics in the colony grown in PDA. Variations in texture and hyphal thickness occurred among culture media: cassava peelings have slightly compact with concentric rings; taro and sweet potato peelings have concentric rings; arrowroot and PDA produced puffy concentric rings. Thin hyphal thickness was observed in both PDA and arrowroot, while the three media have moderately thick to thick characteristics. The linear growth of different culture media formulated from HKW is comparable to PDA, used as the standard culture medium for T. harzianum. The result suggests that the different starch-based HKW may be used as alternative culture media for the commercial production of *T. harzianum*, which may support pests' biocontrol and the farmers' increased yield.

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INTRODUCTION

Worldwide waste generation is escalating due to rapid population growth and urbanization. Over 90% of wastes are openly burned or thrown away in unregulated dumping sites in low-income countries, resulting in environmental consequences and creating a breeding ground for disease vectors, which pose serious health risks to humans (World Bank, 2019). Undeniably, proper and effective waste management is crucial for a livable environment. The fruit and vegetable production process generate significant by-products because plant material is typically susceptible to microbial spoilage (Huang et al., 2010). Vegetable peelings, such as root crops, are often discarded and unimportant. This is also added to the wastes and dumped in open landfills, which could attract insects such as flies that could spread microbes and cause disease. On the other hand, various minerals, elements, and dietary fiber are found in the peel of root crops (Huang et al., 2010). Thus, discarded peels of starchy root crops such as cassava, sweet potato, arrowroot, and taro from household wastes could be transformed into an alternative product such as a low-cost alternative component of culture medium for fungi.

Numerous studies were conducted to determine how to utilize household waste in low-cost media production. The continuous search for less expensive culture media is necessary due to the concerns over the escalating expense of culture media. Numerous alternative media are developed and tested as substitutes for the agar. In Europe, a low-cost medium capable of supporting the growth of the bacterium *P. oryzihabitans* PGP01 at a 25°C medium was made using three waste products generated from potato peels and pulp, tomato seeds, and wheat bran. Growing in this medium preserved its biological activity, demonstrating the expected impact on the growth of the roots (Cantabella et al., 2021).

In Brazil, a study investigates various formulations of culture media derived from plant products to grow microorganisms and produce industrially relevant microbial compounds. Compared to conventional media, these alternative media frequently exhibit comparable microbial growth efficiency and cost of production. In addition to horticulture products, vegetable substrates such as soy, beans, corn, and rice were included in most formulations (dos Santos et al., 2022). In Egypt, agro-industrial waste is investigated for the production of bacterial nanocellulose, and the results are remarkable in terms of physical characteristics and microstructure properties (Abol-Fotouh et al., 2020). Moreover, in India, the nutritional values of kitchen wastes such as vegetable stalks and fruit peels are utilized for alternative media production. Using nine formulations, the growth of bacteria and the effectiveness of pigment synthesis were examined. The findings demonstrated that the growth of *E. coli, Serratia sp.*, and *Pseudomonas sp.* was supported by drumstick formulations containing seed and peel extracts (Jadhav et al., 2018), *Aspergillus*, and *Trichoderma* (Kadam et al., 2017). Temple wastes containing vegetable material were used to prepare microbiological nutrient media to cultivate common bacteria, including *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, and fungi such as *Aspergillus niger*, *Penicillium chrysogenum*, *Saccharomyces cerevisiae*, and *Torulla*. The results were highly encouraging, demonstrating that temple waste microbiological media supported a robust growth of both bacteria and fungi than the standard controlled media (Gurav & Pathade, 2011).

The country is blessed with rich soil and a tropical climate. The farmers produced several crops with economic importance throughout the country. The Philippines, as an agricultural country, has tried to address the inefficient management of waste by creating the Republic Act 9003 also known as Ecological Solid Waste Management Act of 2000, which provides substantial waste reduction and avoidance through recycling, and composting. Before disposal in the appropriate facilities, other methods create necessary intuitional mechanisms and incentives, appropriating funds, and providing penalties to those who violate the act (Aquino et al., 2013). However, solid waste disposal, including kitchen waste, has become a problem in many local government units (LGUs). In the Province of Negros Occidental, the act's implementation has not been fully established, the 3Rs are not practiced, and food service vendors' waste ends up in landfills (Acanto, 2016).

One common microorganism that inhabits the rhizosphere is the *Trichoderma* spp., which is also acknowledged as one of the potential biocontrol agents of soil-related plant pathogens (Guzmán-Guzmán et al., 2019; Harman et al., 2021; Harman, 2011; Harman et al., 2004; Herrera-Estrella & Chet, 2004; Zin & Badaluddin, 2020).

In addition, *Trichoderma* sp. has improved plant development and lessened the severity of various plant diseases due to its application as a biocontrol agent (Nusaibah & Musa, 2019; Poveda, 2021; Rush et al., 2021; Sánchez-Montesinos et al., 2021; Sood et al., 2020; Tyśkiewicz et al., 2022). As a beneficial soil microorganism, *Trichoderma harzianum* can be employed as a biological agent to halt the spread of plant diseases, especially in some significant crops like corn, peanuts, and other legumes.

One of the essential concerns for a sustainable community is its capacity to generate affordable and renewable products from household kitchen waste. These kitchen wastes can be utilized for renewable and environment-friendly biofuels and other by-products (Matsakas et al., 2014). Root crops like cassava, sweet potato, taro, and arrowroot had become the locals' primary food source. Peelings are usually discarded after processing and end up as garbage. To minimize the pollution and build-up of methane gas due to the decomposition of these biodegradable wastes, the researchers wanted to utilize the root crop peelings and develop an alternative culture medium, *T. harzianum*. Furthermore, the researcher investigated alternative culture media using household kitchen wastes for growing fungi such as *T. harzianum* to be used in a laboratory.

MATERIALS AND METHODS

Acquisition of Sample Fungal Organism (Trichoderma harzianum)

The sample organism was acquired at the Regional Soils Laboratory of the Department of Agriculture Region VI, Iloilo City, Philippines. The Agricultural Technologist of the Office of the Provincial Agriculturist, Province of Negros Occidental, confirmed and certified the sample fungus species as *T. harzianum*. The sample organism was brought to Herbanext Laboratories Inc. in Brgy Taloc, Bago City, Negros Occidental, for storage and future use. The samples were stored in agar plates and agar slants in a refrigerator maintained at 4° C.

Preparation of Alternative Culture Media from Household Kitchen Wastes (HKW)

Peelings of cassava, taro, sweet potato, and arrowroot were collected from different households in Brgy. Mandalagan, Bacolod City, Negros Occidental. The samples were packed using clean Ziplock bags and transported to the laboratory for processing. Household kitchen wastes were sliced into small pieces, rinsed severally in clean water, then oven-dried until crisp. About 500g of dried peelings were ground into powder using a mortar and pestle. About 250g of powdered HKW was mixed with 1000 mL distilled water in a 1L beaker and heated to 100°C for one hr. using a hot plate with constant stirring. The mixture was filtered using a vacuum filter, and the filtrate was obtained and weighed. Using a calibrated digital weighing scale, the starch yield recovered from 500g of each HKW dry sample are the following: taro peelings yielded 70g of starch, cassava peelings had a starch yield of 120g, arrowroot peelings had a starch yield of 185g, and 151g of starch was recovered from sweet potato peelings. The filtrate of each crop peeling was then used as the main component of a culture medium for the growth of *T. harzianum*.

Formulation of Alternative Culture Media from Household Kitchen Wastes (HKW)

The alternative culture media was formulated using the formulation suggested by Aryal (2022); each alternative culture medium contains 20% HKW filtrate, 2% dextrose, and 2% agar in every specific amount of water. Table 1 shows each culture medium's formulation from different household kitchen waste sources: cassava, taro, sweet potato, and arrowroot peelings. For Cassava Dextrose Agar, 120g of cassava filtrate was mixed with 12g of dextrose, 12g of agar, and 600 mL of distilled water. About 70g of taro filtrate, 7g of dextrose, 7g of agar, and 350 mL of distilled water were mixed to make Taro Dextrose Agar. About 185g of arrowroot filtrate was mixed in 18.5g of dextrose, 18.5g of agar, and 925 mL of distilled water for Arrowroot Dextrose Agar. For Sweet Potato Dextrose Agar, 151g of sweet potato filtrate was mixed with 15.1g of dextrose, 15.1g of agar, and 755 mL of distilled water. In preparing the Potato Dextrose Agar, 200g of PDA was mixed with 1000 mL of distilled water; PDA is a positive control in the study. A 1000 mL Erlenmeyer flask containing each solution was thoroughly mixed and autoclaved for 15 minutes at 121°C; the flask was sealed with a cotton plug and wrapped in aluminum foil. After autoclaving, about

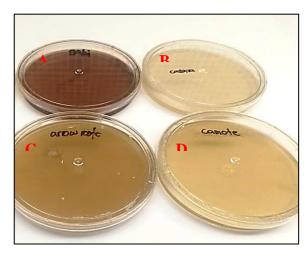
20 mL of each alternative culture media mixture was poured into each sterile Petri dish and allowed to cool at room temperature to harden and be ready for use. Extra agar plates were stored in a sterile refrigerator maintained at 4°C.

The appearance of alternative culture media made from the different vegetable peelings of household kitchen wastes is presented in Figure 1. Taro culture medium (A) appeared to be maroon in color, cassava culture medium (B) was opaque, arrowroot (C) appeared to be light brown, and sweet potato (D) appeared to be very light brown.

Table 1

Formulation of Each Alternative Culture Medium

Alternative Culture Medium	Filtrate/Extract	Dextrose	Agar	Distilled Water
Cassava	120g	12g	12 g	600 mL
Taro	70g	7 g	7 g	350 mL
Arrowroot	185g	18.5g	18.5 g	925 mL
Sweet Potato	151g	15.1 g	15.1 g	755 mL



- A. Taro Culture Medium
- B. Cassava Culture Medium
- C. Arrowroot Culture Medium
- D. Sweet Potato Culture Medium

Figure 1. The appearance of different alternative culture media made from vegetable peelings of HKW

Fungal Growth Radial Assay

The fungal culture media containing various household kitchen wastes formulation were examined if they could support *T. harzianum*. Pure cultures of *T. harzianum* were retrieved from the stock cultures and allowed to acclimatize at room temperature for one hour. A previously sterilized scalpel was used to cut a colony block (2mm x 2mm dimension) from the mother plate. It was placed on plates containing a culture medium formulated with (1) cassava peel powder, (2) taro peel powder, (3) sweet potato peel powder, (4) arrowroot peel powder, and (5) premixed PDA. A calibrated Vernier caliper was used to measure the fungal radial growths on the second, fourth, sixth, and eighth days of incubation on the plates containing the prepared culture media. The plates were incubated at 28°C for eight days. The radial growth assay was performed with three replicates of each HKW sample. The treatment groups were various household kitchen wastes, and the pre-mixed PDA served as control.

Safety Issues and Safety Protocol

Trichoderma species are unusual but emerging human pathogens that could result in infections due to specific risk factors (Sandoval-Denis et al., 2014). T. harzianum was properly handled inside a BSL2-certified biosafety

laboratory. The analyst was trained in safely handling the microorganism based on the Center for Disease Control and Prevention (2009) guidelines. The analyst adhered to standard laboratory safety procedures, including disinfecting laboratory surfaces, using proper personal protective equipment, minimizing aerosol production, and handling splash hazards associated with manipulating plant extracts and *T. harzianum* cultures. Contaminated pipet tips, inoculating needles, and all Trichoderma cultures were disposed of through autoclave sterilization. Also, an aseptic technique was observed throughout the study to prevent contaminants that may affect the result.

Analysis of Data

Visual observation of each colony of the experimental and control groups was done on the 8th day after inoculation to identify the top-view colony characteristics, such as the colony surface color, texture, and hyphae thickness. The morphological description for colony characterization of *T. harzianum* cultured in various HKW was based on Shah & Afiya (2019). Mean and Standard Deviation was used to determine the average linear growth rate of four experimental and control set-up replicates.

Using computer-generated software, a two-tailed One-way Analysis of Variance (ANOVA) was used to determine the significant difference in the linear growth rate of *T. harzianum* between the experimental and control groups.

RESULTS AND DISCUSSION

Top View Colony Characteristics of T. harzianum on different Culture Media

It was found that all alternative culture media supported the growth of *T. harzianum* in varying degrees and characteristics. The morphological features of *T. harzianum*, such as color, texture, and hyphal thickness, differ depending on the culture medium. The top-view colony characteristics of *T. harzianum* after eight days of inoculation on different culture media are shown in Table 2. The surface color of *T. harzianum* in the culture medium of cassava peelings is white moss green. In contrast, the surface color of taro and sweet potato peelings is white to brownish green. Arrowroot has a white to light green surface color, and Potato dextrose agar has a white to blue-green color. Some variations were observed: a) cassava peelings were slightly compact with concentric rings; b) taro and sweet potato peelings were close with concentric rings, and c) in arrowroot and potato dextrose, agar produced puffy and concentric rings. In the case of hyphal thickness, the moderately thick hyphal consistency was observed in cassava peelings, while taro and sweet potato peelings had thick hyphal thickness. A thin hyphal was observed in potato dextrose agar.

The different variations in the top view colony characteristics of *T. harzianum* in alternative cultural media made from various household kitchen wastes were due to the main component of the culture medium. It could be noted that the color of the culture medium depended on the color of the material it was made of, like taro and sweet potato agar plates, which are yellow to orange. The *T. harzianum* colony's color may also depend on the agar plate's location. The observation was coherent with the Sharma & Pandey (2010) results that the fungal growth, colony characteristics, and sporulation are influenced by culture medium in different ways. The top-view colony characteristics can also be observed in Figure 2.

Table 2

Culture Medium	Surface Color	Texture	Hyphal Thickness
Cassava Peelings	White to moss green	Slightly compact with concentric rings	Moderately thick

International Research Journal of Science, Technology, Education, and Management Volume 2, No. 4 | December 2022

Taro Peelings	White to brownish	Compact with concentric rings	Thick
Sweet Potato Peelings	green White to brownish	Compact with concentric rings	Thick
Arrowroot Peelings	green White to light green	Puffy with concentric rings	Thin
Potato Dextrose Agar	White to blue-green	Puffy with concentric rings	Thin

Top View Colony Characteristics of T. harzianum in Different Culture Media

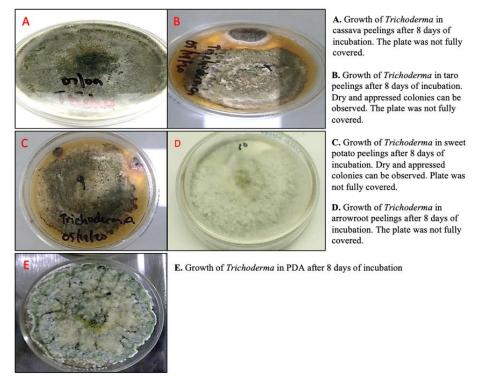


Figure 2. Top View Colony Characteristics of T. harzianum

The average colony radial growth of three replicates of *T. harzianum* was calculated and tabulated in Table 3. On day 2, the highest growth was found in PDA with a mean radial growth of 6.1 mm, and the lowest was obtained from the taro peelings with a radial growth of 4.5 mm. A radial growth of 16 millimeters was recorded in PDA on the fourth day, followed by 14.3 millimeters in sweet potato peelings, 12.5 millimeters in arrowroot, and 11.5 millimeters in cassava peelings. Taro peelings had the smallest amount of radial development, 9.9 mm. On the 6th day, the maximum radial growth was obtained in PDA at 22.5 mm, followed by sweet potato peelings at 18.9 mm, arrowroot at 18.1 mm, and taro peelings at 16.3 mm. The minimum growth was obtained in the cassava peelings with 14.4 mm.

With a mean radial growth of 41.4 mm on the eighth day, PDA continued to yield the highest growth, followed by sweet potato peelings (35.1 mm), arrowroot (34.3 mm), and taro peelings (33.7 mm). The cassava peels contained the smallest amount of radial development (30.9 mm).

The highest growth rate of *T. harzianum* was in PDA (M=21.43 mm, SD= 12.89), followed by sweet potato peelings (M=17.93 mm, SD=10.90), arrowroot peelings (M=17.55 mm, SD=10.68), and taro (M=16.1 mm, SD= 10.99). While in the case of cassava peelings, *T. harzianum* slowed down on day six and day eight, resulting in a growth rate of (M=15.43 mm, SD=9.57), which was the lowest among the other culture media. The results show a variation in the growth rate of *T. harzianum* in different cultures based on their standard deviations. The result also implies that PDA is still the best option for growing fungi like *T. harzianum* and produces optimum growth for *T*.

harzianum; however, agar plates containing household kitchen wastes could also support fungal growth. The result suggests that starch could also be obtained from discarded peelings of root crops such as cassava, taro, sweet potato, and arrowroot, promoting the growth of fungal species. According to Otache et al. (2017), based on their proximate and mineral analysis, cassava peels are rich in nutrients, mainly carbohydrates. Dusuki et al. (2020) reported that significant values of carbohydrate content were found in sweet potato peels and cassava peels. Figure 2 shows the growth rate pattern of different culture media used in the study.

Table 3

Test Groups	Day 2	Day 4	Day 6	Day 8	Mean	SD
Cassava Peelings	4.9	11.5	14.4	30.9	15.43	9.57
Taro Peelings	4.5	9.9	16.3	33.7	16.1	10.99
Sweet Potato Peelings	5.0	14.3	18.9	35.1	17.93	10.90
Arrowroot Peelings	5.3	12.5	18.1	34.3	17.55	10.68
Potato Dextrose Agar	6.1	16.0	22.5	41.4	21.43	12.89

Linear Growth Rate (in mm) of T. harzianum in Different Culture Medium

*Note: Results presented were the mean of the three replicates of each culture medium.

Figure 3 shows the linear growth rates of different culture media made from various household kitchen wastes (cassava, taro, sweet potato, and arrowroot peelings). The graph shows an exponential linear growth rate of different culture media from the start of inoculation (Day 0) until the last day of observation (Day 8). The graph also shows that PDA had the highest linear growth rate since it was a standard culture medium for fungi, followed by sweet potato peelings, arrowroot peelings, and taro peelings. The lowest linear growth rate among the culture media was the cassava peelings. Among the alternative culture media made from household kitchen wastes, it could be suggested that sweet potato peeling is most favorable to the growth of *T. harzianum*. Huang et al. (2010) indicate that the sweet potato peels were high in protein and starch. This may suggest the favorable growth of *T. harzianum* in the culture medium made of sweet potato peelings.

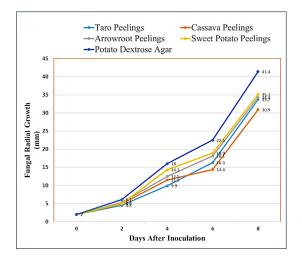


Figure 3. Growth Pattern of T. harzianum in different culture media

The difference in the Average Linear Growth of T. harzianum among the Different Culture Media Using Household Kitchen Wastes

Table 4 presents the difference in the linear growth rate of *T. harzianum* in a culture media made from various peelings of starch-based vegetable household kitchen wastes. The result shows no significant difference in the linear growth rate of *T. harzianum* among different culture media using household kitchen wastes, $F_{(4,15)} = 0.138$, p = 0.97.

The result also shows that the culture media made from various household kitchen wastes could be comparable with the Potato Dextrose Agar, the standard culture medium for fungi like *T. harzianum*.

The results showed that fungi could grow on the formulated culture media. The finding is consistent with Akharaiyi & Abiola (2016), who demonstrated the utilization of alternative culture media for fungal growth. The fact that the fungi grew on the prepared media suggests that the peels used to make the media had the nutrients needed for fungal growth. Their analysis of the relative starch contents of pineapple peel, yam peel, and plantain peel revealed that the peelings contained, respectively, 78.32%, 60.87%, and 61.02% starch.

Also, culture media containing high carbohydrate sources supported the growth of fungi. The result shows that taro, cassava, sweet potato, and arrowroot peelings could support the growth of *T. harzianum* when used as a low-cost alternative to PDA. The result agrees with Ferreira Nogueira et al. (2018) work, which showed a result of high amylose content (35%) of arrowroot starch. Fakir et al. (2012) revealed that cassava contains a significant amount of starch; from 100g of fresh tuber, 20.41g of starch was extracted. The culture medium made from sweet potato had the highest growth rate compared with other culture media made from arrowroot, taro, and cassava. Also, it shows that a culture medium made from sweet potatoes is the best alternative for growing fungi in the laboratory (Kitahara et al., 2017). This finding could result from the high starch content of the sweet potato.

Table 4

One-Way Analysis of Variance Results for the Difference in the Linear Growth Rate of T. harzianum Among the Different Culture Media Using Household Kitchen Wastes

	Sum of Squares	df	Mean Square	F-ratio	р
Between Groups	90.227	4	22.557	0.129	0.07
Within Groups	2445.845	15	163.056	0.138	0.97
Total	2536.072	19			

CONCLUSION AND RECOMMENDATION

There were variations in the colony characteristics of *T. harzianum* found in different culture media used in the study. Depending on the main component of the culture media, *T. harzianum* colonies exhibit a range of different properties. Cassava, taro, sweet potato, and arrowroot peelings can be used as an alternative material to produce culture media for the production of *T. harzianum*, supporting and favoring its growth. An alternative, cheaper culture medium for *T. harzianum* can be introduced using waste products from the kitchen that include starch.

Further research on optimization and standardization of the formulation of culture media made from these HKW for commercialization may help the farmer-beneficiaries enhance production yield. Farmers may consider the formulation of culture media for the growth of *T. harzianum* as a biological agent to control for pests of their crops which could reduce the usage of harmful pesticides that is expensive and harmful to the environment. Organic household kitchen wastes such as vegetable peelings could be utilized as a valuable resource, such as culture media for microorganisms useful in agriculture as biocontrol agents.

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