

Contemporary Food Waste Formulation; Alternative Culture Media for *Trichoderma* species

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ABSTRACT

Researchers and industry aim to utilize food waste for positive reasons. Processing cassava generates a lot of waste, which is widely regarded as a major source of pollution in the environment. Alternative media for *Trichoderma* species development were developed in this study. Agar media were made independently from the peels of cassava and yam. Using potato dextrose agar (PDA) as a control, *Trichoderma harzianum* and *Trichoderma viride*, which were isolated from dumpsite soil, were cultivated on the prepared media. Over the course of four (4) days, the growth rate of *Trichoderma* spp. was measured and varied from 6 to 8 mm. On yam peel dextrose agar (YPDA), *Trichoderma harzianum* and *Trichoderma viride* grew more readily than on cassava peel dextrose agar (CPDA). As of day, four (4), *Trichoderma harzianum* had grown by 62.2% in CPDA, 88.9% in YPDA, and 100% in PDA, whereas *Trichoderma viride* had grown by 48.9%, 66.7%, and 100% in CPDA, YPDA and PDA respectively. The findings showed that, *Trichoderma* species were able to proliferate when exposed to yam and cassava peels. Therefore, to reduce the expense of growing fungi using synthetic media, cassava and yam peels could be used as an alternative fungal medium instead of being thrown away as garbage that will contaminate the environment.

Keywords: Fungi Media, Cassava Peels, Yam Peels, *Trichoderma* spp. Waste.

Introduction

Food waste nowadays is generated in a bulk amount which is not at all sustainable to the economy of the country; hence methods of reusing it have been continuously developed and improved (Ramirez *et al.*, 2020). Interestingly, the scientific community has put great efforts in the development of methodologies for the use of various types of food waste, whether of animal or vegetable origin, in order to obtain new products or compounds of interest or to optimise processes (Leira *et al.*, 2019; Martínez-Montañaño *et al.*, 2021).

The nutrients required for microbial development are provided by culture media (Gamit *et al.*, 2023). For making these media, which can be used for microbial growth or for other reasons, such as selective, differential media and media used to promote the production of microbial compounds, a variety of raw materials are available (Rouf *et al.*, 2017).

As a source of fibres, polyphenols, and bioactive compounds in general, organisation and management of fruit and vegetable residues for human and animal consumption (Ramirez *et al.*, 2020), and use in the creation of alternative culture media, agro-industrial waste is extremely diverse and has the potential to be applied in many different fields (Costa *et al.*, 2019; Ramirez *et al.*, 2020). In institutions with insufficient financial resources, practical microbiology classes and scientific researches are hampered in underdeveloped nations by the high cost of culture medium (Jadhav *et al.*, 2018). Similarly, some researches have indicated that vegetable-based formulations may be useful as substitute (non-commercial) culture media since these substrates offer a variety of nutrients, including proteins, necessary for the growth of microorganisms (Santos *et al.*, 2021). These techniques, which are still in development, attempt to increase sustainability by using waste materials or agro waste components not frequently utilised to make food for humans, including vegetable peels and stems (Gamit *et al.*, 2023).

Trichoderma comprises a genus of filamentous fungi, saprophytic, avirulent and opportunistic plant symbionts, inhabiting mainly the soil, rhizosphere, and endosphere of plants (Ali *et al.*, 2021; Guzman-Guzman *et al.*, 2023). *Trichoderma* spp. has been (mostly) considered as non—pathogenic and opportunistic plant symbionts, which can colonize plant roots, establishing a beneficial interaction with their hosts mediated by *Trichoderma* effector proteins and hormonal crosstalk in exchange for plant-derived sugars (Mendoza-Mendoza *et al.*, 2018; Guzman-Guzman *et al.*, 2019; El Enshasy *et al.*, 2020a).

By virtue of their versatile mechanisms of action and the capability of adaptation to different environments, *Trichoderma* spp. have been used to control plant diseases caused by fungi, nematodes and insects that deprive crops in different conditions such as greenhouse, field and post-harvest (Ali *et al.*, 2021). Besides, *Trichoderma* spp. offer additional advantages to the agriculture such as increase in plants' photosynthetic capability and yields, efficient absorption of nutrients and abiotic stress tolerance (Sood *et al.*, 2020).

As established by the United Nations in the Sustainable Development Goal 2.4, by 2030 sustainable and resilient agricultural practices that increase the productivity, reinforce the capacity for adaptation to climate change, and progressively improve the soil quality must be implemented to nourish the growing worldwide population and diminish the perils of hunger (United-Nations 2015, as cited in Ali *et al.*, 2021). Considering the exposed features, *Trichoderma* spp. are promising tools to contribute to this objective (Ali *et al.*, 2021). In educational and research institutions with financial constraints for the procurement of conventional cultural media, the goal is to offer a scientific basis for, and support the production and use of, these culture media (Gamit *et al.*, 2023).

This study therefore aimed at formulating alternate culture media made using plant-based waste ingredients for the growth of *Trichoderma* species. The specific objectives of this study were to isolate and identify *Trichoderma* species from soil sample,

Preparation of growth formulation media from cassava and yam peels, and culturing and evaluation of the growth of *Trichoderma* species on formulated media.

Materials and Methods

Isolation and identification of *Trichoderma* species from soil sample

A modified method of isolation and identification of *Trichoderma* species demonstrated by Dania (2019) was adopted. One gram of soil was weighted using electronic compact scale SF -400 C and dissolved in 100mL distilled water amended with 2% citric acid in a sterilized test tube. An aliquot of 1mL of the solution was added to 15mL malt extract agar (MEA) in each Petri dish.

Isolates were further purified on *Trichoderma* selective medium (TSM) with the following constituents (grams per litre of distilled water): 3.0g chloramphenicol, 0.25g p-dimethylaminobenzenediazo sodium sulfonate, 0.3g pentachloronitrobenzene, 0.2g rose bengal (tetrachlorotetradiodofluorescein), 0.15g agar: 0.5g MgSO₄.7H₂O, 0.2g K₂HPO₄, 0.9g KC1, 0.15g NH₄NO₃ and 1.0g glucose.

Trichoderma isolates were identified according to their colour, shape. To appreciate the microscopic feature of the fungi isolated, lactophenol cotton blue was dropped on a clean glass slide, little growth of the fungus was removed with a sterile inoculating needle, and the preparation was covered with a clean cover slip and examined under the microscope with x10 magnification. Microscopic examination and morphological characteristics were noted and compared with existing authorities.

Preparation of media from yam and cassava peels

A modified media preparation method as demonstrated by Escalante *et al.* (2022) was adopted.

Cassava and yam peels were sliced separately into small pieces with the aid of a sterile scalpel, rinsed severally in clean water, then oven-dried using GenLab thermal engineers DC 125 serial No. 15L169 oven until crisp. About 500g of each dried peeling were ground into powder using a mortar and pestle.

About 250g of powdered samples weighted using electronic compact scale SF -400 C were mixed with 1000mL distilled water in a 1L beaker and heated to 100°C for 1h using a hot plate Sensor Disc HP-5 Taisite USA, with constant stirring. The mixture was filtered into a different 1L beaker using a muslin cloth, and the filtrate obtained was weighed using the same weighing balance. The filtrates of cassava and yam peelings were then separately used as the main component of a culture medium for the growth of *Trichoderma* species.

Preparation of growth formulation media

Growth formulas were prepared following Escalante *et al.* (2022) procedure. Each growth formula medium contained 20% substrate filtrate, 2% dextrose, and 2% agar in every specific amount of water. Therefore, in 1000mL of water in three conical flasks, 200g of filtrates, 20g dextrose and 20g agar were added separately forming Cassava Peel Dextrose Agar (CPDA) and Yam Peel Dextrose Agar (YPDA).

In preparing the Potato Dextrose Agar (PDA), 200g of PDA was mixed with 1000mL of distilled water; PDA was used as a positive control in this study. Separately 1000mL Erlenmeyer flask containing each solution was thoroughly mixed, each flask was sealed with a cotton plug, wrapped in aluminium foil and autoclaved for 15min at 121°C. After autoclaving, about 20mL of each prepared culture media mixture was poured into separate sterile Petri dishes and allowed to cool at room temperature to harden and be ready for use.

Growth evaluation of *Trichoderma* species on formulated media

The prepared culture media containing growth formulation was examined if they could support *Trichoderma* species growth. Pure cultures of *Trichoderma* spp. were retrieved from the stock culture and allowed to acclimatize at room temperature for one hour. A sterilized scalpel was used to cut a colony block (2mm x 2mm dimension) from the mother plate. Each was then placed on plates containing a culture medium formulated with cassava peel powder (CPDA), yam peel powder (YPDA), and pre-mixed PDA. Plates were cultured in duplicates. The cultured plates were incubated at 28°C for 8 days during which period a transparent ruler was used to measure the fungal radial growths on the second (2nd), fourth (4th), sixth (6th), and eighth (8th) day of incubation and recorded in centimeter (cm). The radial growth assay was performed with two replicates of each substrate sample. The treatment groups were various tuber peels substrate, and the pre-mixed PDA served as control.

Results

Macroscopic and microscopic characteristics of the *Trichoderma* species isolated are presented in Table 1. While the growth of the isolates *Trichoderma harzianum* and *Trichoderma viride* on the Cassava peels dextrose agar, Yam peels dextrose agar, and on Potato dextrose agar are presented in Plates 1 to 6.

Table 1: Macroscopic and microscopic identification of *Trichoderma* species isolates

Isolate code	Macroscopy on PDA	Microscopy	Tentative fungi
RM7	Green mycelia with white aerial hyphae	Separate, distinct, septate, irregularly branched conidiphores. Short-skittle shape, bulged at the middle, narrower and singly phialides.	<i>Trichoderma harzianum</i>
RM8	Colony was smooth surface, cottony white mycelial mat with aerial hyphae. Dark yellowish mycelia.	Green conidia distributed. Long, swollen in the middle and horn-shaped phialides	<i>Trichoderma viride</i>

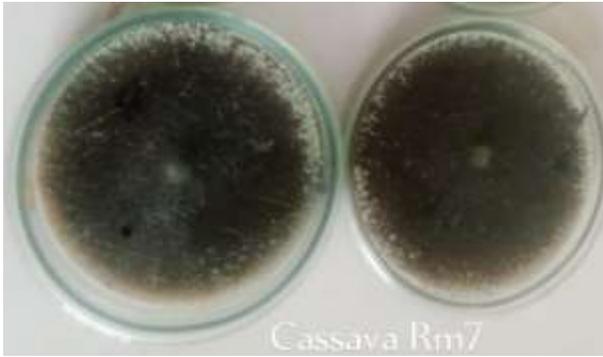


Plate 1: *Trichoderma harzianum* on CPDA Day 4



Plate 4: *Trichoderma viride* on CPDA Day 4

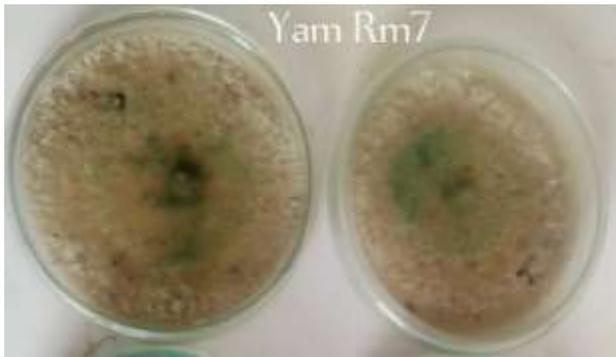


Plate 2: *Trichoderma harzianum* on YPDA Day 4

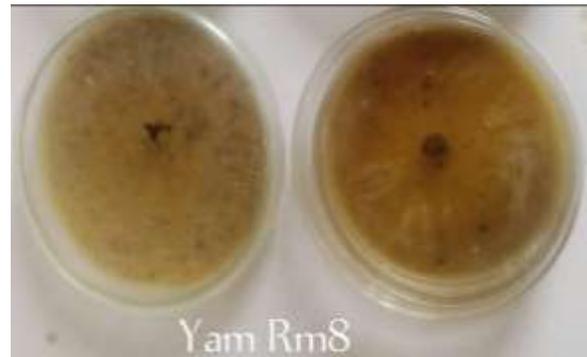


Plate 5: *Trichoderma viride* on YPDA Day 4

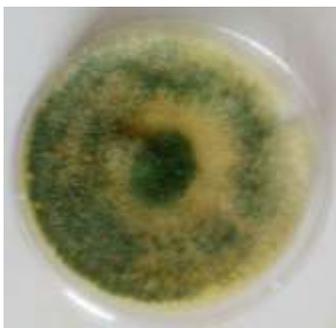


Plate 3: *Trichoderma harzianum* on PDA Day 3



Plate 6: *Trichoderma viride* on PDA Day 3

Key: CPDA = Cassava peels dextrose agar, YPDA = Yam peels dextrose agar, PDA = Potato dextrose agar

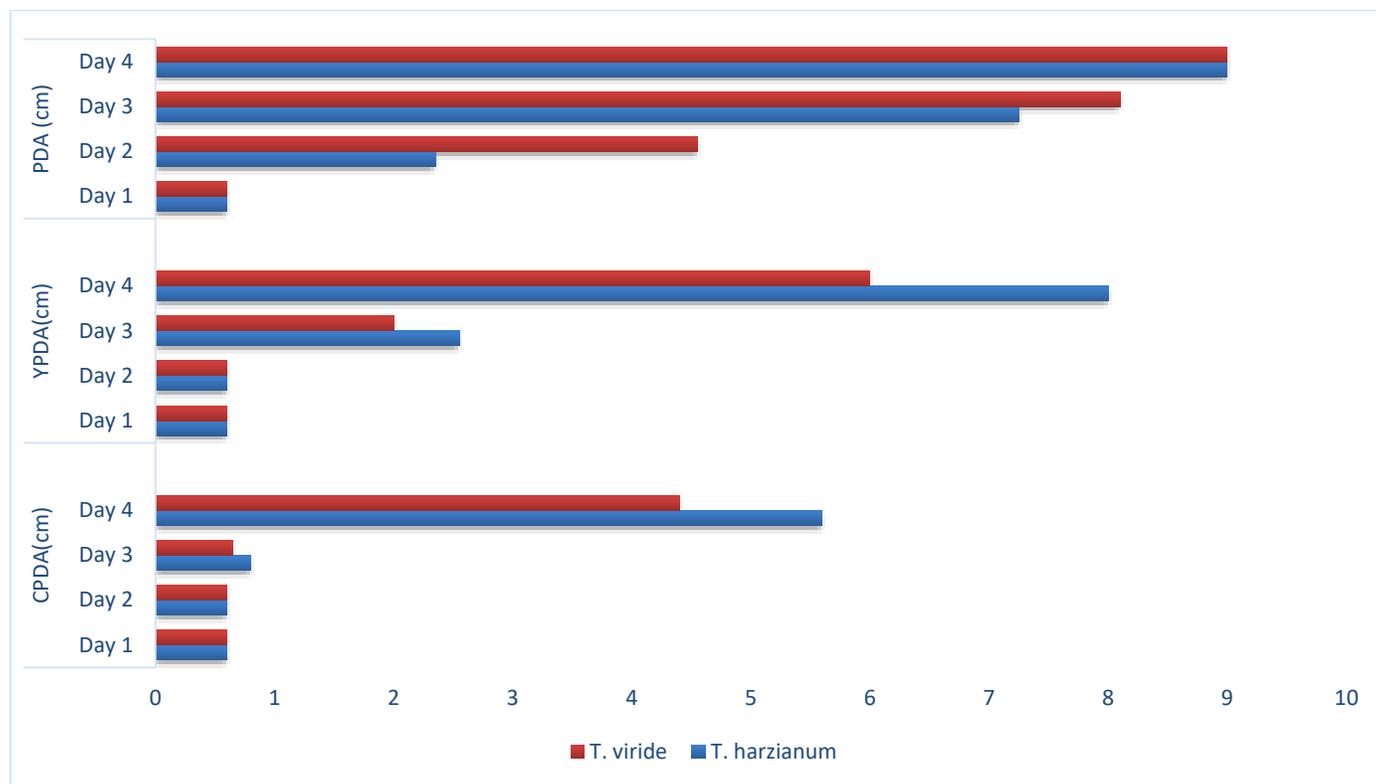


Figure 2: Growth of *T. harzianum* and *T. viride* on Cassava peel agar, yam peel agar and Potato dextrose agar.

Discussion

The *Trichoderma* species used in this study were isolated from dumpsite soil. *Trichoderma* spp. are ubiquitous in nature and very easy to isolate from samples on culture media. The isolates were *Trichoderma harzianum* and *Trichoderma viride*. Other researchers have also isolated *T. harzianum* and *T. viride* from soil samples (Saravanakumar and Wang, 2020; Yassin et al., 2021; Alwadai et al., 2022).

Trichoderma harzianum and *Trichoderma viride* both grew well on cassava peel dextrose agar. They both started showing radial growth on day 3 after inoculation. On day 3, *T. harzianum* grew from the initial 0.6cm to 0.8cm radially, while *T. viride* grew from the initial 0.6cm to 0.65cm. The growth of *T. harzianum* on cassava peel formula in this study agrees with the result 0.2cm to 1.15cm on day 4 obtained by Escalante et al. (2023) from their study, when they grew experimentally *T. harzianum* on cassava peel formula. In another study, cassava peels have been used successfully to grow *Trichoderma brev* T069 (Zhang et al., 2022).

Also, *Trichoderma harzianum* and *Trichoderma viride* both grew well on yam peel dextrose agar. They both started showing radial growth on day 3 after inoculation. On day 3, *T. harzianum* grew from the initial 0.6cm to 2.5cm radially, while *T. viride* grew from the initial 0.6cm to 2cm. there is no previous study in the past 10 years using yam peels formulated media to grow *Trichoderma* species; however, there are reports from studies on yam peels formulated media used to grow other microorganisms. *Saccharomyces carlsbergensis* have been grown on formulated yam peel extract agar (Ugah and Nwoba, 2018), various formulations of yam peels were used to cultivate *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus* sp., *Staphylococcus aureus*, *Streptococcus* sp., *Klebsiella pneumoniae*, *Proteus mirabilis*, *Candida albicans*, *Rhizopus* sp., *Penicillium* sp., *Aspergillus* sp.

Cultural comparison of *Trichoderma harzianum* and *T. viride* on cassava peel dextrose agar (CPDA), yam peel dextrose agar (YPDA) and potato dextrose agar (PDA) revealed that the fungi grew well on CPDA, grew better on YPDA and grew best on PDA.

Trichoderma harzianum recorded 62.2% growth in CPDA, 88.9 % in YPDA and 100 % in PDA while *Trichoderma viride* recorded 48.9 % in CPDA, 66.7 % in YPDA and 100 % in PDA as at day four (4). This is as a result of difference in nutrient composition of the media. While Potato dextrose agar is constituted with the essential nutrients required for fungi grow, crude peels of cassava and yam can also be utilized for fungi growth.

Future studies will focus on evaluating the nutritional and phytochemical contents of cassava and yam peels that supports or inhibits fungal growth, physical parameters such temperature and pH also the breed of the cassava or yam.

Conclusion

The result obtained from this study revealed that cassava and yam peels support the growth of *Trichoderma harzianum* and *Trichoderma viride*. Therefore, cassava and yam peels rather than being discarded as waste could be used as an alternative fungal media in order to minimize cost of growing fungi using synthetic media.

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