Molecular Profiling Ecofriendly *Trichoderma* Biological Control Agent of Yam Tuber Microbial Rot in Northern Nigeria

Onyinyechi Ndimele Akomah-Abadaike and Grace Amaefula Elenwa

**ABSTRACT**

This research on isolation and identification of yam rot pathogen fungi from North central Nigeria was carried out to investigate some fungal species associated with yam rot using white yams (*Dioscorea rotundata*) obtained from Adamawa State, Benue State and Plateau State (Jos) Nigeria. Seven fungi species which cause yam rot were isolated during this study, namely: *Aspergillus flavus*, *Aspergillus niger*, *Penicillium citrinum*, *Penicillium echinulatum*, *Penicillium purpurogenum*, *Trichoderma spp* and *Trichophyton spp*. *Trichoderma yunnanense* strain RCBBR_GA1 OP8008361 was identified molecularly. Pathogenicity test carried out confirmed these organisms as the pathological agent of the rot. *A. niger* statistically has the highest frequency of occurrence (100%). The least encountered fungal species were the *P. citrinum* (16.6%), *P. purpurogenum* (33.3%) and *Trichophyton spp* (33.3%). Percentage growth inhibition of *A. flavus*, *A. niger*, *P. citrinum*, *P. echinulatum*, *P. purpurogenum*, *Trichoderma spp* and *Trichophyton spp* was carried out and they showed varying degree of inhibition. However, *Trichophyton spp* shows the highest growth of inhibition at 96 hours (41.4%) and 120 hours (73.7%). *Trichoderma* exhibited the highest control of the isolates (80 mm). *Trichoderma* inhibited mycelia extension growth of *A. flavus* (25 mm), *A. niger* (25 mm), *P. citrinum* (23 mm), *P. echinulatum* (23 mm), *P. purpurogenum* (24 mm) and *Trichophyton spp* (22 mm) at 96 hours. The result shows that *Trichoderma* was able to inhibit the growth of the pathogenic fungi, which are associated with yam rot. Fungal isolates from yam rot were examined morphologically and microscopically and the nature of rot was varied. It is recommended that enzymes from *Trichoderma* should be extracted and applied for reduction of microbial rot of yam tubers.

**Keywords**: Pathogenicity, rot, *Trichoderma yunnanense*, yam tubers.

1. INTRODUCTION

Yam is a root vegetable that grows underground and is edible. Additionally, it is a climbing plant’s tuberous root that is commonly cultivated in tropical and subtropical nations. It belongs to the Dioscorea genus. Yams are monocots that share genetic ancestry with grasses, lilies, and perennial herbaceous vines. They are grown for their starchy tubers in a variety of temperate and tropical climates, including West Africa and South America. Yam is a staple food and a superior source of carbohydrates (Bantilan, 2019; Obidiegwu et al., 2020; Oloruntoba & Sridhar, 2015). Yams are a key agricultural and cultural product in West Africa, where 95% of the world’s yam crop and over 70% of the total is gotten from Nigeria (Anwadike, 2019; Akinbo et al., 2015). They live on about seven million hectares of planted land (Ramírez & García, 2019; FAOSTAT, 2021).

‘Rot’ is a significant problem that restricts the supply of yams and can result in significant losses. Rot losses have a severe impact on yam seed stockpiled for planting, farmers’ and dealers’ income, and food security. Rot degrades yam tubers’ quality, rendering them unattractive to consumers. Pathogenic fungi like *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium chrysogenum*, *Penicillium oxalicum*, *Trichoderma viride*, and *Rhizopus nodosus* are the main microbes responsible for yam rot (Gwa & Ekefan, 2017).
Although there are certain difficulties, the use of chemicals like fungicides has shown to be successful in controlling those microorganisms that cause rot. Chemicals are not only expensive and hard to come by, but they also harm the environment. Therefore, the hunt for non-chemical alternatives to chemical ones for controlling yam disease has been sparked by the need for safe and non-polluting solutions. Biological management of plant pathogens has been suggested as a viable treatment. Trichoderma are environmentally benign biological control agents that help manage plant diseases while reducing the need for chemical fungicides (Puyam, 2016).

According to Holzlechner et al. (2016), Trichoderma species are soil-borne filamentous saprophytes that can grow on a variety of substrates, including plants, animals, and soil. They are renowned biocontrol agents for fungi that are transmitted through soil (Waghunde et al., 2016). Trichoderma has been discovered on plant roots, in decaying wood, and in many kinds of soils (Puyam, 2016). Trichoderma participates in the breakdown of plant waste and nitrogen absorption in soil (Govarthanan et al., 2018). To combat many fungal plant infections such as Alternaria, Pythium, Sclerotinia, Fusarium, and Botrytis, the majority of Trichoderma species show antagonistic properties (Mukhopadhyay & Kumur, 2020; Kumar et al., 2018). The study was aimed at the isolation and identification of fungi causing yam rot in three different northern states, which are Benue State, Adamawa State, and Plateau State (Jos), through macroscopic and microscopic methods. Its objectives were (i) Identification of microorganisms associated with rot of yam tubers. (ii) Determination of the biocontrol or antagonistic activity of Trichoderma on rot-causing fungi (iii) Molecular identification of the Trichoderma species with biocontrol potential.

2. Materials and Methods

2.1. Sample Collection

A total of thirty-six (36) yam samples, six (6) healthy yam samples, and six (6) unhealthy (spoilt) yam samples were collected from three different north states each which are Adamawa State, Benue State, and Plateau State (Jos), Nigeria. The samples were packaged in a sterile polyethylene bag and transported to the laboratory (Emadavistics Medical and Research Laboratory at Alakahia, Port Harcourt) for immediate microbial analysis.

2.2. Sample Preparation and Isolation of the Fungi Isolates

Healthy and diseased yam tissues from the different north-central states in Nigeria (Benue State, Adamawa State, and Plateau State) were washed under running tap water and cut into various sizes and put into different Petri dishes for the isolation of fungi. The sample materials were sterilized, incubated at room temperature for 24 hours, and then subjected to microscopic examination. Fungi were isolated and identified using a combination of macroscopic and microscopic methods.
TABLE I: MORPHOLOGICAL AND MICROSCOPIC FEATURES OF FUNGAL ISOLATES FROM YAM ROT

<table>
<thead>
<tr>
<th>S/N</th>
<th>Species of fungi</th>
<th>Morphological features (colour and texture)</th>
<th>Microscopic features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aspergillus flavus</td>
<td>Green, yellow velvety surface, cream, surrounding</td>
<td>Septate hyphae with long conidiospore.</td>
</tr>
<tr>
<td>5.</td>
<td>Penicillium purpurogenum</td>
<td>Greenish smooth surface with red, purple ending with cracked red reverse.</td>
<td>Septate, bronchial conidiophores with phialides sponge-like arrangement.</td>
</tr>
<tr>
<td>6.</td>
<td>Trichoderma spp</td>
<td>Blue-yellow and green patches, wooly colonies with creamy surrounding.</td>
<td>Septate hyphae with separate chlamydospores exceeding from the conidiospores.</td>
</tr>
</tbody>
</table>

water to remove surface soil, debris, and other contaminants. The yam samples were cut approximately at the range of 2 mm × 2 mm with a sterile scalpel, and the cut pieces were first surface sterilized by dipping completely in a concentration of 5% sodium hypochlorite solution for 2 minutes and rinsed in four successive changes of sterile distilled water (SDW) for further microbial analysis. Infected yam tissues were later picked onto sterile filter paper using sterile forceps and then blotted with filter paper for 2–3 minutes in the laminar air-flow cabinet. The dried infected tissues were aseptically removed from the healthy tuber surfaces using a five-millimetre cork borer. For inoculation, a 5 mm-diameter mycelia agar plug of a 5-day-old fungus culture was employed.

The holes made in the yam tubers were filled with these fungus plugs. The margins of the new yam tissues were sealed with petroleum jelly. For the control trials, the same process was followed, except discs of PDA that had not yet been inoculated were inserted into the holes made in the tubers instead.

5. EVALUATION OF ANTAGONISM USING DUAL CULTURE ON AGAR PLATES

The dual culture approach was used to conduct the antagonism assay on Potato Dextrose Agar (PDA) in Petri dishes. On the same dish, 5 mm-diameter mycelial plugs of the antagonist (Trichoderma spp.) and the pathogenic fungi isolated from the yam were inoculated, with the antagonist and pathogenic fungus spaced 6 cm apart. Two days before and two days after the pathogen was inoculated, the antagonist was plated on Petri dishes containing 15 ml of sterile PDA at the same time as the pathogens.

For 192 hours, paired cultures were incubated at 30 °C room temperature. The antagonist against the pathogen was substituted with a PDA plug in the control plates. Measurement of radial mycelia growths of the Trichoderma spp., Aspergillus flavus, Penicillium citrinum, Aspergillus niger, Penicillium purpurogenum, Penicillium echinulatum, and Trichophyton spp. in dual culture and Trichoderma spp. in control plates was done after three days of inoculation on a 24-hour interval beginning from the 72 hours up to the 192 hours of incubation at ambient room temperature (30 °C ± 5 °C).

6. MOLECULAR IDENTIFICATION OF FUNGAL ISOLATE

With the help of a Zymo Quick DNA fungal and bacterial extraction kit, fungus genomic DNA was extracted. The ITS4: TCCTCCGCTATTGATATG and ITS5: GGAAGTAAAAGTCGTAACAGG primers
were used to identify fungi. The ingredients in the PCR cocktail mix are 2.5 ul of 10× PCR buffer, 1 ul of 25 mM MgCl2, 1 ul of forward and reverse primers, 1 ul of DMSO, 2 ul of 2.5 mM dNTPs, 0.1 ul of 5 ul/ul Taq DNA polymerase, and 3 ul of 10 ng/ul DNA. Using 13.4 ul of nuclease-free water, the total reaction volume was increased to 25 ul. Denaturation at 94 °C for five minutes is followed by 36 cycles of annealing at 54 °C for 30 seconds throughout the PCR cycle and elongation at 72 °C for 45 seconds. Afterwards, the temperature was maintained at 10 °C indefinitely after a last elongation stage at 72 °C for 7 minutes. On 1.5% agarose electrophoresis gels stained with Safe View, amplified fragments were made visible. The DNA ladder utilized is the Hyper ladder and microscopically analysed, many types of rot were prevalent fungal species with 50% occurrence.

When yam rot fungal isolates were morphologically and microscopically analysed, many types of rot were discovered. At 80 mm, *Trichoderma* showed the greatest virulence. Due to the pathogen’s capacity to exploit the nutrition in yam as a substrate for growth and development, the pathogenicity test revealed that the pathogenic fungi seeded in the yam tubers produced rot. This outcome is comparable to that of Okigbo *et al.* (2015) study on the fungi connected to Nigerian yams, which involved *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Penicillium oxalicum,* and *Penicillium chrysogenum.* According to this study, *Aspergillus niger* occurs most frequently (statistically, 100% of the time), while *Penicillium citrinum* is the least common fungal species (Table II). Two-third of the yam sample has above fifty per cent (50%) of the microbial rot causing organisms. Due to *Aspergillus niger*’s capacity for biodeterioration, yam tuber rots can occur either in the field or during storage, which has resulted in significant financial losses, a decline in the market value of field product, and low farmer income. Some writers supported my conclusions, such as Okigbo *et al.* (2015) who claimed that *Aspergillus niger* was the most harmful fungus on *Disocore a rotundata.* The dual culture results showed that *Trichoderma* spp. can inhibit the growth of other fungi’s mycelia regardless of when the antagonist is introduced. The antagonist (*Trichoderma* spp.) and the pathogenic fungi were inoculated on the same dish with the antagonist (*Trichoderma* spp.) and pathogenic fungi using 6 cm from each other, and the *Trichoderma* spp. was able to antagonize other pathogenic organisms (Fig. 1). The mycelia extension growth of *Aspergillus flavus* (25 mm), *Aspergillus niger* (25 mm) (Fig. 3), *Penicillium citrinum* (23 mm) (Fig. 4), *Penicillium echinulatum* (23 mm), *Penicillium purpurpureum* (24 mm), and *Trichophyton* spp. (22 mm) was suppressed by *Trichoderma,* which was isolated from yam tubers, at 96 hours. The maximum growth inhibition is seen in *Trichophyton* spp. at 96 hours (41.4%) and 120 hours (73.7%). *Aspergillus niger* has the least percentage growth inhibition at 96 hours and *Aspergillus flavus* exhibits the least at 120 hours (both 11.4% and 29.7%). (Table III). *Trichoderma* sp inhibitory effect using Sangoyomi scale (Gwa *et al.,* 2019) was effective for five (5) of the isolated

<table>
<thead>
<tr>
<th>S/N</th>
<th>Isolates/Inoculated fungi</th>
<th>Rot (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Trichoderma</em> spp</td>
<td>80 mm</td>
</tr>
<tr>
<td>2.</td>
<td><em>Trichophyton</em> spp</td>
<td>70 mm</td>
</tr>
<tr>
<td>3.</td>
<td><em>Penicillium echinulatum</em></td>
<td>65 mm</td>
</tr>
<tr>
<td>4.</td>
<td><em>Aspergillus niger</em></td>
<td>55 mm</td>
</tr>
<tr>
<td>5.</td>
<td><em>Penicillium purpurpureum</em></td>
<td>51 mm</td>
</tr>
<tr>
<td>6.</td>
<td><em>Penicillium citrinum</em></td>
<td>45 mm</td>
</tr>
<tr>
<td>7.</td>
<td><em>Aspergillus flavus</em></td>
<td>30 mm</td>
</tr>
</tbody>
</table>

were among the microorganisms that were isolated for this investigation (Table I). This is consistent with the findings of Shiriki *et al.* (2015, 2019) and Anwadike, 2019 who noted the presence of some of the above-mentioned species, including *Aspergillus niger.* Ajayi and Olorundare (2014) reported in their research that *Aspergillus niger* is the most prevalent fungal species with 50% occurrence.
rot causing pathogen being above 50% inhibition which moderately effective against one below 50% (Fig. 1).

*Trichoderma* are facultative anaerobes with a wide range of abilities, a high level of rhizosphere competence, a profusion of root colonizers, and a global outlook. Additionally, they are avirulent, opportunistic plant symbionts. Extracellular proteins and fungitoxic compounds are frequently produced by them (Puyam, 2016). The isolates with the most effective control are *Trichoderma* (80 mm), whereas the isolate with the worst suppression is *Penicillium citrinum* (30 mm).

*Trichoderma* yunnanense strain RCBBR_GA1 OP800836.1 was the *Trichoderma* strain that was molecularly identified. *Trichoderma* yunnanense OP80036.1, with a similarity of 99.82%, is its closest GenBank match (Fig. 2). The isolate has a mean concentration of 194.2 ng/ml and a purity of 1.96 at A260/280 according to the NanoDrop spectrometry parameters. *Trichoderma* exhibits blue-yellow and green patches with fuzzy colonies and a creamy background, according to morphological and microscopic findings.

8. Conclusion

In some regions of Nigeria, fungi are the principal cause of rot in yam tubers. *Aspergillus flavus*, *Aspergillus niger*, *Penicillium citrinum*, *Penicillium eheimatum*, *Penicillium purpurogenum*, and *Trichophyton* spp. are among the pathogenic fungi that have been isolated. The use of biological antagonists that are safe for the environment can prevent fungus from growing and surviving, which opens up new options for managing fungal diseases in yams. When additional fungal organisms were introduced on the same dish as the antagonist in this investigation, *Trichoderma* yunnanense strain RCBBR_GA1 OP800836.1, it was able to prevent their proliferation. To lessen the microbiological rot of yam tubers, *Trichoderma* enzyme extraction is advised.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

References


