Isolation and Identification of *Trichoderma* species From South-South Geopolitical Zone of Nigeria

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ABSTRACT

Yam rot caused by fungal pathogens has been of great concern to farmers in Nigeria and farmers of South-South Geopolitical zone of Nigeria are not exempted of this yam spoilage plight. This study was carried out on yam cultivated and harvested in the South-South of Nigeria to isolate and identify eco-friendly biological control agent (*Trichoderma* sp.) that could be used to control yam rot-causing fungi. The yam samples were purchased from local farmers in Rivers state. *Trichoderma* sp. was isolated from healthy yam peels, while the fungal pathogens were isolated from the rotting part of yam by serial dilution and then pour plating. The fungi isolated and identified as pathogens were *Penicillium oxalicum*, *Penicillium roquefortii*, *Aspergillus niger, Aspergillus flavus*, *Rhizopus nigricans* and *Rhodotora* sp., while the isolated biological control agent was identified as *Trichoderma harzianum*. An antagonism test of the *T. harzianum* against all the pathogens was conducted by co-culture method and the result in percentage inhibition were *P. oxalicum* (22.00%), *P. roquefortii* (26.50%), *A. niger* (49.80%), *A. flavus* (40.00%), *Rhodotora* sp. (31.58%) and no antagonism against *R. nigricans* (0%). The result obtained in this study revealed that *Trichorderma harzianum* has a broad inhibitory capability against yam rot-causing fungal pathogen. Therefore, *T. harzianum* can be effectively utilized by farmers to safely control the menace of yam rot caused by fungal pathogen.

Keywords: *Trichoderma harzianum*, Antagonism, Eco-friendly, Pathogens*, R. nigricans*

INTRODUCTION

Yam is a staple in the South-South region of Nigeria, hence its spoiling is significant economically. Trichoderma is a genus of fungus that is widespread in various types of soil and, as a result, is easily detected in rhizospheres and on plant tubers. Trichoderma species are influenced by a number of variables, such as microclimate, substrate availability, and intricate

E. O. Nwaichi and F. O. Nduka (eds.), *Proceedings of the 6th Biennial International Conference for Organization for Women in Science for the Developing World Nigeria (OWSD 2023)*, Advances in Biological Sciences Research 37,
https://doi.org/10.2991/978-94-6463-306-1_6

ecological relationships (Hoyos-Carvajal & Bissett, 2011). Due to its antagonistic (inhibitory) activity against a variety of plant diseases and growth promotion in crop plants, the genus Trichoderma has grown significantly in importance over the past few decades (Filizola et al., 2019).

Agrochemicals are currently used extensively to combat pest and disease threats. However, the use of such compounds is linked to a number of issues, including the emergence of resistant infections and environmental contamination (Druzhinina et al., 2010; Taylor et al., 1999), in addition to the high production costs that are reflected in the price of the finished products. In addition to aiding in environmental conservation, biological control stands out as a substitute for less harmful agricultural development (Filizola et al., 2019). As a substitute measure, it has become more and more popular due to its relative simplicity, cleanliness, and ease of use.

Through the use of natural enemies like microbes in the ecosystem, biological control is an effective and environmentally friendly way to lower the prevalence of disease (Dania, 2019). The goal of biological control agents (BCAs) is to significantly lower the prevalence of disease while also enhancing the ability of hormones that promote growth to promote plant development and ultimately yield (Pascal et al., 2017).

Presently, antagonistic fungi play a significant role in the use of biological control agents (BCAs), which is a well-established fact. In some circumstances, BCAs even replace chemical counterparts in this regard (Chet, 1993; Whipps and Lumsden, 2001). Due to its capacity to provide environmentally friendly disease control, particularly when included in an integrated pest management strategy, biological control of plant pathogens has gained substantial recent attention as a different disease management technique (Tondje et al., 2007). In several studies (Harman et al., 2004; Holmes et al., 2004; Rai and Singh, 1980; Samuels et al., 2006; Sanogo et al., 2002; Scharen and Bryan, 1981), isolates of Trichoderma species have been shown to be mycoparasitic on a variety of fungi.

Trichoderma species are intriguing candidates to study because they account for approximately 50% of the fungal BCA market, primarily as soil or growth enhancers (Whipps and Lumsden, 2001). Numerous species in the genus Trichoderma have the ability to parasitize ascomycetes and even species that are phylogenetically related, which makes them unusual (Shan, 2009; Kubicek et al., 2019). Trichoderma species T. reesei, T. virens, and T. atroviride have their genomes examined, and the results indicated that mycoparasitism is an intrinsic trait of Trichoderma; yet, these species also exhibit significant nutritional versatility: Trichoderma species have an exceptional propensity to create enzymes that cause lysis of the host cell, which promoted their use as a biocontrol agent against plant pathogenic fungus in addition to acting as mycoparasite (Kubicek et al., 2019). According to Dania (2019), Trichoderma species exhibit an exceptional propensity to create enzymes that lyse the target pathogens' mycelia and result in the formation of secondary metabolites or antibiotics. With more than 1500 registered preparations worldwide (Verma et al., 2007), they are the most common natural fungicides used in commercial farming (Dania, 2019).

Controlling yam spoiling due to rot brought on by microbes is necessary. Chemical techniques, which are frequently hazardous to people, are used to control spoilage organisms. This calls for the adoption of simple, inexpensive, non-toxic biological control techniques like Trichoderma antagonism against spoilage germs. This study set out to find Trichoderma species or strains with antagonistic properties toward the fungus responsible for yam rot. Its goals include (i) isolating Trichoderma and (ii) isolating the fungus responsible for yam rot. (iii) Trichoderma species were tested for their ability to combat isolated fungi that cause rot.

MATERIALS AND METHOD

Isolation of *Trichoderma* species from healthy yam peels

Trichoderma species were isolated using a modified procedure by Ajayi and Olorundare (2014). A peel from a healthy yam sample was collected, dipped in 5% sodium hypochlorite, rinsed in distilled water, and then placed on sterile potato dextrose agar with the peel's bark touching the medium. For 48 to 72 hours, the plate was incubated at room temperature in order to allow for a decent amount of fungal growth. Various fungal growths on the plate were incubated for 48–72 hours before being subcultured onto sterile PDA medium and incubated again.

Isolation of yam rot-causing fungi

Using the pour plate approach, Willey et al. (2014)'s rot-causing fungus were isolated from PDA. 1 gram of each sample of rotten yam was taken at the point where the rot had advanced using a sterile knife. The sample was then serially diluted by first putting 1 g of it into a test tube with 9 mL of distilled water, and then putting 1 mL of the resulting combination into another test tube with 9 mL of distilled water to create dilution 2 (10-2). Then, 20 mL of molten PDA was poured to fill the base of the plate after 1 mL of 10-2 dilution had been deposited with a sterile pipette onto a sterile Petri dish solidified; it was kept at room temperature for 48–72 hours in order to encourage enough development. All of the rotten yam samples were isolated in this manner.

Identification of fungal isolates on culture plates

The fungal isolates were identified using lactophenol cotton blue stain and plate morphology (see culture plates of identified isolates in appendices). Different colonies' colony morphology, including size, shape, spore type, and coloration, was studied and noted. The fungal isolates were prepared as a wet mount in lacto-phenol cotton blue on a clear microscope slide with cover slips. It was examined using the microscope's high power (x40) and subsequently low power (x10) objectives.

Antagonistic test of *Trichoderma* species against isolated fungi causing yam rot

Following the dual culture/co-culture method of Otadoh et al. (2011), antagonistic activity of isolated Trichoderma species against isolated fungal pathogens of yam rot was investigated in vitro. Test fungus (pathogens) were transferred onto two sterile PDA plates at a distance of 0.5 cm from the center of the plates using a sterile cork borer with a diameter of 1 cm. The antagonist (Trichorderma sp.) was then placed on one of the plates, 0.5 cm from the center and in opposition to the test fungi. All six of the isolated pathogens were set up using the same method. The culture plates were then incubated at 25 °C for five days. Both the pathogen and the biocontrol agent (Trichoderma sp.) showed colony expansion, and the pathogen's radial growth was noted every day until the fifth day (day 5) following the immunization. The following relationship was used to compute the % inhibition (antagonism of Trichoderma sp. against pathogens):

$$P.I = \frac{C_5 - T_5}{C_5} \times 100$$

Where:

P.I = Percentage inhibition of mycelial growth.

C_5 = Radial growth (cm) of pathogen in control plate on day 5 after inoculation.

T_5 = Radial growth (cm) of pathogen in co-culture with *Trichoderma harzianum* on day 5 after inoculation.

3. RESULT AND DISCUSION

Table 1: Identified fungal isolates

Isolate	Colonial morphology	Microscopy	Probable fungi
A	Blue-greenish spherical shaped, rough raised center with white margin	Long hyphae with brush-like round conidiophores	*Penicillium oxalicum*
B	Greenish-gray, velvety texture with regular margin	Separate, septate, bearing a cluster of branches, phialides born on cylinder branches and arranged brush-like head	*Penicillium roqueforti*
C	Black, powdery	Conidiophores smooth, tall and brownish, aseptate, globose	*Aspergillus niger*
D	Yellow-green surface and reverse golden-red	Septate hyphae, rough conidiophores	*Aspergillus flavus*
E	Brown, cottony, filamentous colony,	Unbranched conidiophores, aseptate, rounded columella.	*Rhizopus nigricans*
F	Yellow swarming colony	Singly budding cells	*Rhodotora* sp.
G	Green surface	Separate, distinct, septate, irregularly branched, not verticillate	*Trichoderma harzianum*

Table 2: Antagonism of *Trichoderma harzianum* against isolated pathogens

Fungal pathogens	Day 1		Day 2		Day 3		Day 4		Day 5	
	C_1	T_1	C_2	T_2	C_3	T_3	C_4	T_4	C_5	T_5
Penicillium oxalicum	0.50	0.50	2.15	1.71	3.10	2.20	3.60	2.80	4.00	3.12
Penicillium roqueforti	0.50	0.50	2.24	1.81	3.15	2.35	3.70	2.84	4.40	3.23
Aspergillus niger	0.50	0.50	2.91	1.06	4.61	2.10	5.68	2.70	6.40	3.21
Aspergillus flavus	0.50	0.50	2.53	1.90	4.12	2.45	5.40	2.91	6.00	3.60
Rhizopus nigricans	0.50	0.50	1.74	1.78	2.31	2.30	4.82	4.81	5.20	5.20
Rhodotora sp.	0.50	0.50	1.11	1.00	1.93	1.71	2.46	1.99	3.80	2.60

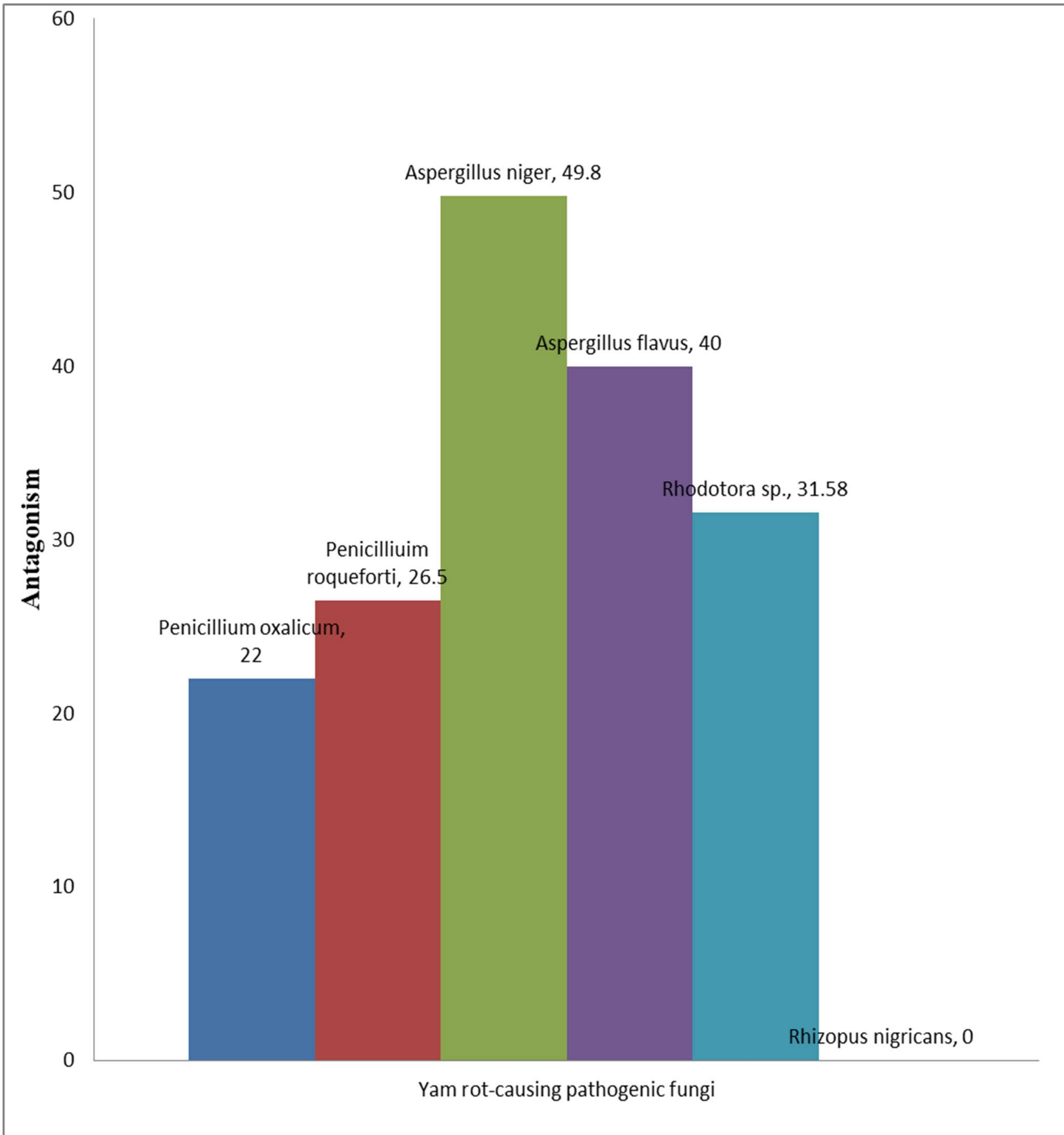

Fig. 1: Percentage inhibition of T. harzianum against fungal pathogen

Fig. 2: *Aspergillu oxalicum*

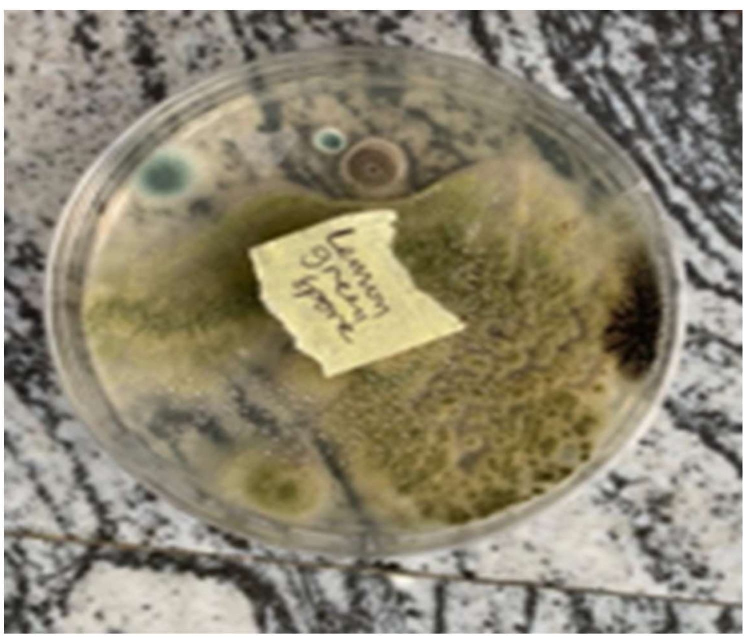

Fig.3: Isolated Aspergillus flavus

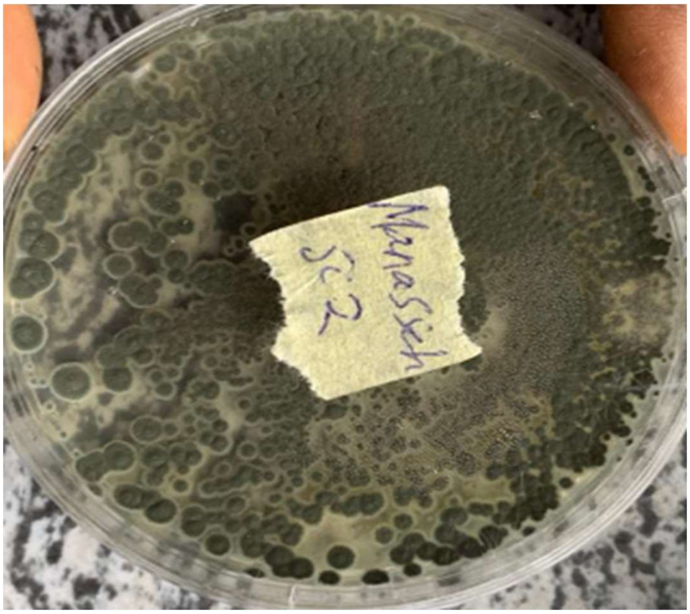

Fig 4.: *Penicillium oxalicum*

Fig.5: Rhizopus nigricans

Fig. 6: *Trichoderma harzianum*

Discussion

The detected fungal isolates are listed in Table 1, along with the macroscopic and microscopic characteristics that were used to identify them. According to the findings, Aspergillus niger, Aspergillus flavus, Penicillium oxalicum, Penicillium roquefortii, Rhodotora sp., and Rhizopus nigricans were the pathogens and Trichoderma harzianum was the isolated antagonist.

Trichoderma harzianum showed the maximum antagonism (49.80%) against Aspergillus niger, followed by Aspergillus flavus (40.0%), Rhodotora sp. (31.58%), Penicillium roqueforti (26.50%), and P. oxalicum (22.0%), with the lowest antagonism. Rhizopus nigricans was not negatively affected by T. harzianum. These results are consistent with those from Jat and Agalave's (2013) investigation of the antagonistic properties of Trichoderma species against fungi that are found in oilseeds.

Conclusion

According to the study's findings, Trichorderma harzianum possesses a wide range of inhibitory properties against the fungus responsible for yam rot. Farmers can therefore use T. harzianum to safely control the threat of yam rot brought on by a fungal infection.

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