

Biological Control of Post Harvest Rot-Inducing Fungi of White Yam Tubers (*Dioscorea rotundata* Poir.) in Storage with Antagonistic Biofungicides

Anuagasi, C. L., Okigbo, R. N., and Anukwuorji, C. A.

Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

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Corresponding Author: **Anuagasi, C. L** | E-Mail: (canuagasi@gmail.com)

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ABSTRACT

An antagonistic study was carried out to assess the potential inhibitory capability of Biofungicides of *Bacillus subtilis*, *Pseudomonas syringae*, *Trichoderma harzianum*, and *Trichoderma viride* as biocontrol agents against rot-inducing fungi of white yam (*Dioscorea rotundata* Poir.) tubers in storage. Rot-inducing fungi and Biofungicides used for control were isolated using standard methods. A pathogenicity test was also carried out to ascertain whether the fungi induced rot or not in healthy yam tubers. culture method was used to evaluate the effects of biofungicides. Biofungicides each paired with the pathogenic test fungi served as treatments. *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Aspergillus flavus*, and *Penicillium Spp* were the fungi consistently isolated from rotted yam samples. All fungi identified were pathogenic hence, induced rot in healthy yam tubers after 14 days of inoculation. The most virulent was *Aspergillus niger*. The degree of rot was evaluated by calculating the percentage inhibition by the Biofungicides. Inhibition of rot ranged from 13.16 ± 0.070^a - 48.67 ± 0.045^c % in *Bacillus subtilis* to 12.95 ± 0.288^a - 40.16 ± 0.058^a % in *Trichoderma viride* when paired with the pathogenic test fungi. This study revealed that the Biofungicides have potential to control rot in post-harvest yams. Overall, *Bacillus subtilis* was the most effective Biofungicides in controlling the pathogenic test fungi. Inference of this study showed that the use of Biofungicides as biocontrol agents is an economically viable way of suppressing post-harvest rot of white yam.

Keywords: Biological control, Antagonistic, Fungal rot, Post-harvest, Biofungicides, Storage

INTRODUCTION

Yam (*Dioscorea* Spp) belongs to the family Dioscoreaceae. It is a herbaceous annual climbing plant with edible underground tubers¹. Yams have been reported to comprise of about 600 species worldwide^{2,3}. Ngo-Ngwe *et al.*⁴ reported that yam is considered a famine food and plays a prime role in the food habits of small and marginal rural families and forest-dwelling communities during the food scarcity periods. Viruel *et al.*⁵ pointed out that it is recognized as the fourth most important tuber crop after cocoyam, cassava, and sweet potatoes and contributes about 10% of the total root and tubers production around the world.

White yam (*Dioscorea rotundata* Poir.) according to Onwueme^{6,7} is the most important species of yam in West Africa. It is a monocotyledonous, perennial herb cultivated for the consumption of its starchy tuber⁸. Okigbo *et al.*⁷ reported that yam tuber is chiefly the main useful part of the crop. Its prepared in diverse ways before consumption, it maybe pounded, roasted, boiled or fried. Opara and Nwokocha⁹ reported that yam is essential in food security especially in the tropics this is because it has a relatively longer shelf life than most crops.

Yam rot according to Okigbo *et al.*¹⁰ is the biological degradation of yam tubers in storage facilities or in yam barns. It reduces both the quality and quantity of yam tubers whether in storage or before consumption. Amusa *et al.*¹¹, Anukwuorji *et al.*¹² reported that yam rots or deterioration of cultivated yam usually starts in the soil and progresses to other stages of growth

and processing within the value chain. Tuber rot or spoilage occurs even when there is no any external sign of symptom or infection in the infected tuber. Yam spoilage is common in an environment with very high moisture content in the atmosphere; this is because high humidity and temperature of 25-39°C encourage the growth and proliferation of the rot inducing microorganisms¹³.

Biofungicides according to Dicklow¹⁴ are formulations of living organisms that are used to control the activity of plant pathogenic fungi and bacteria. Biofungicides are microorganisms that are applied to the soil or foliage to prevent or control pathogenic bacterial or fungal infections in plants and plant roots. Dicklow¹⁴ reported that these microorganisms produce a wide range of antibiotic substances, parasitize and compete with other fungi, and induce localized or systemic resistance in plants. Antagonistic microorganisms according to Mokhtar and Aid¹⁵; Gwa *et al.*¹⁶ can compete with the pathogen for food, stops the growth and proliferation of pathogenic organisms either by mycoparasitism or by secreting antibiotics or toxins.

Biological control method using Biofungicides has several advantages when compared to chemical products and are far more economical than plant extracts. Okigbo¹⁷ reported that biological control of plant disease is has beneficial effects over chemical pesticides. Anuagasi *et al.*¹⁸ reported that biocontrol agents are ecofriendly, mild on non-target organisms and decompose easily in the environment. Furthermore, Okigbo and Ikediugwu¹⁹ reported instances where biological control has

showed to last longer than chemical control substances in its effects and in most cases does not require regular applications. This research is aimed at evaluating the antagonistic potential of some Biofungicides on storage rot-inducing fungi.

MATERIALS AND METHODS

Preparation of Culture Media

Potato Dextrose Agar (PDA) and Nutrient agar (NA) were used as the media for the growth and maintenance of the fungal and bacterial isolates. The PDA and NA were prepared in accordance with the manufacturer's instructions. The prepared media was transferred to a Pyrex media bottle and sterilized in the Autoclave at 121°C, the pressure of 15 (Psi) for 15 minutes²⁰. After sterilization, the medium was allowed to cool to about 45°C before aseptically dispensing into 15ml aliquots to sterile glass Petri dishes which were allowed to cool down and gel. After solidification, each agar plate was aseptically wrapped round with masking tape until needed for use. These plates were stored in the refrigerator.

Isolation of Fungal Pathogens from Rotted Yam Tubers

The method was described by Ritichie²¹, and modified by Okigbo *et al.*⁷. Yam tubers with symptoms of rot were surface sterilized by immersing totally in a 10% concentration of sodium hypochlorite solution for 2 minutes. This step was to remove surface contaminants. Rotten yam tubers were washed and rinsed in sterile distilled water and surface sterilized again by cleaning with 70% ethanol-soaked cotton wool. The yam tubers were cut open with a surface sterilized kitchen knife to reveal the boundary area between the healthy tubers and the rotten side. Inocula were taken as pieces cut off with flamed scalpel (5mm by 5mm) in size. The yam pieces were then placed on sterile paper towels in the Laminar Air Flow Cabinet to dry for 5 minutes. Some of the Inocula were transferred directly into sterile PDA plates, while others were first teased out in sterile distilled water. Inocula or loopfuls of the resulting suspension was transferred into sterile PDA plates and spread evenly over the surface. All the plates were incubated 'up side down' in an incubator at room temperature (28 to 32°C) for 2 to 5 days. The plates were however observed daily for fungal growth and development. When growth has established, sub-cultures were prepared using Inocula from the different organisms in the mixed culture as obtained.

Sub-culturing and Purification of the Fungal Isolates Pathogenic to Yam Tubers

The test fungi were purified by transferring hyphal tips from the colony edges to fresh plates of PDA with the aid of flame-sterilized blades²⁰. These plates were incubated in the incubator at 28 to 32°C for 2 to 5 days and observed daily for fungal growth and development. Several subculturing was done thus, purifying the mixed culture plates. Stock cultures were prepared by using the surface sterilized fungi which were consigned into McCartney slant bottles of acidified PDA and stored in a refrigerator for characterization and further studies^{7, 22}.

Identification and Characterization of Fungal Isolates

Identification of fungal isolates was based on the morphological or structural features as seen on the culture plates as well as slides viewed under the compound microscope. Microscopic examination involved slide mounts of test isolates stained with Lactophenol Cotton Blue Stain. Morphological or structural features as observed from each isolate were matched against

those present in standard manual or identification guides²³.

Determination of Percentage Frequency of Occurrence of the Fungal Isolates

This was done to determine the incidence of occurrence of the different fungal isolates associated with yam tubers. The total number of each isolate was obtained against the total number of all the isolates in all the samples screened. Frequencies of occurrence of different fungal isolates were therefore determined using methods described by^{24, 10}. Number of times each fungus was encountered was recorded and the percentage frequency of occurrence was calculated using the formula described by Ebele²⁵ thus:

$$\frac{\text{Number of times a fungus was encountered} \times 100}{\text{Total fungal isolations}}$$

Pathogenicity Test

A pathogenicity test was carried out to ascertain that the fungal isolates from rotted yam samples were able to induce rot or otherwise on healthy yam tubers. The methods of Okigbo and Odurukwe²⁶; Okigbo and Emeka¹³; Anukwuorji *et al.*¹² were adopted. Healthy yam tubers were washed with tap water to remove debris or soil. According to Ritichie²¹ the yam tubers were surface sterilized to expunge surface contaminants by immersing the tubers in 10% concentration of Sodium hypochlorite for 2 minutes and rinsing twice in sterile distilled water. To further remove contaminants, the yam tubers were surface sterilized with 70% ethanol solution. The tubers were then placed on sterile paper towels in the Laminar Air Flow Cabinet to dry for 20 minutes. A sterile cork borer (5mm diameter) was used to bore cones (holes) into the yam tubers at marked points. The cylindrical fleshy portions that were removed from the tubers at each point were kept in sterile Petri dishes. Inocula from the growing culture of each test isolate was introduced into the hole made and the reserved cylindrical part was carefully replaced after inoculation. Part of the tuber removed earlier was cut off to make up for the thickness of the agar inoculum. The replaced core and edges at the point of inoculation was tightly sealed with petroleum jelly to prevent contamination and labelled accordingly. A control experiment was set up by inoculating another tuber with 1ml of sterile distilled water without the fungal isolates. After inoculation, all the tubers were incubated for 14 days at a temperature of 28 to 30°C and examined daily for evidence of rot such as discoloration, softening, offensive odour, etc. After 14 days, the tubers were carefully cut open along the line of inoculation to reveal the main portions of the tubers which were then examined for the extent of rot. Where positive, the length and girth of the rotted area and those of the entire tubers as revealed were measured and recorded.

Isolation, Purification and Identification of Bacterial Antagonist (Biofungicides)

Isolates of *Bacillus subtilis* and *Pseudomonas syringae* with accession numbers, KM972670 and KT887197.1, respectively were obtained using the methods of Okigbo (2002); Okigbo and Emeka (2010) from the topsoil of a farm at NRCRI, Umudike, and from naturally infected tomato fruits showing typical bacterial speck disease symptoms collected from an open market in Umuahia. A ten fold serial dilution (w/v) of the soil in sterile distilled water was first heated to 100°C for 10 min in a water bath. A sample (0.1 ml) of 10³ dilutions in the water of the soil

was spread on yam dextrose agar (YDA) and incubated at room temperature (28 to 30°C) for up to 48 hour during which characteristic colonies of *Bacillus* developed. Isolates were subjected to preliminary microbiological analysis and were further identified using molecular characterization. The tomato fruits were rinsed thrice with sterile distilled water and uniformly segmented. The dissected tomato fruits were surface sterilized with sodium hypochlorite (2% solution) for 3 to 5 minutes followed by rinsing with sterilized distilled water thrice. Subsequently, the tissues were pulverized in 2 ml potassium phosphate buffer (0.05 M) followed by incubation at room temperature for 10 min. Afterwards, the homogenate was streaked on nutrient agar (NA) medium plates with a sterilized inoculating loop (Cheesbrough, 2004). Plates were incubated at 27°C for 48 hours and the germinated bacterial colonies were observed. Bacterial colonies were purified by transferring a single colony culture to a new nutrient agar medium plate to gain the pure bacterial culture that was maintained at 4°C for in glass slants containing NA medium for further studies.

Isolation, Purification and Identification of Fungal Antagonist (Biofungicides)

Trichoderma harzianum and *Trichoderma viride* with accession numbers OR227920.1 and ON208727 respectively were isolated from the surface of spoilt tomato fruits collected from an open market in Umuahia town using the method of Okigbo and Emeka (2010). Each was placed in a 500ml beaker containing 200ml sterilized distilled water placed on a rotary shaker at 100rpm for 12 hours. Afterward, 0.1ml of suspension was taken from the beaker, spread on Potato Dextrose Agar (PDA) plates and incubated at ambient temperature (28±2°C) for 24 hours for colonies to develop. Sub-cultures were made to obtain pure isolates. The isolates were identified to species level by molecular characterization, physiological and morphological standard methods.

In vitro Inhibition of Fungal Isolates by Biofungicides

A modified dual culture technique after Ferreira *et al.*²⁷; Gwa and Abdulkadir²²; Gwa and Ekefan²⁸ was adopted. Determination of the zone of inhibition was done using the dual culture technique. A 2-day old Biofungicide isolate was streaked on one side of PDA or NA in a 9cm Petri dish and each pathogen was inoculated at 5cm away at the opposite side and incubated at 28±2°C. Three replicates of each were made. Control was prepared by inoculating only the pathogen on PDA or NA without being challenged. Percentage inhibition of fungal growth was calculated. The zones of inhibition were measured using a well-calibrated transparent meter rule and recorded.

In Vivo Effects of Biofungicides on Rot Pathogens of Yam Tubers

As part of the precautionary measures to reduce contamination, the healthy yam tubers were washed to remove soil particle and debris. The tuber was rinsed with 10% concentration of sodium hypochlorite for 5 minutes. This was further cleaned with sterile distilled water and sterilized with 70% ethanol. The healthy yam tubers were carefully placed on sterile paper towels in a Laminar Air Flow Cabinet for ease of drying. The methods of Gwa and Ekefan²⁸ were adopted. Treatments comprising the pathogenic fungal isolates, each paired with four biological antagonists (Biofungicides) were set up to determine the effect on rot in yam tubers. *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtilis*, and *Pseudomonas syringae* served as the

antagonist while the fungal isolates served as the control. Each of the Biofungicides was matched with the pathogenic fungal isolate and the yam tubers were inoculated separately. For each treatment, one litre of the pathogenic fungi in liquid medium was mixed with one litre of the biological antagonist in a liquid medium and stirred for 5 minutes. A pin bar (sterile) was used to scratch each yam tuber, starting from the proximal end to the distal end. The injured yam tuber was completely submerged in the dispersed medium for 10 minutes. The yam tubers were then removed and transferred into a moist white nylon bag. To create a conducive environment for the test fungi/pathogen, the nylon bags were tied with a rubber band and punctured. The nylon bags containing the inoculated yam tubers were placed on benches at room temperature for 42 days. The control experiment was set up with yam tubers dipped in pathogenic fungi in a liquid medium. The treated tubers were checked for rot by cutting it at the point where it was injured. To ascertain the degree of rot/damage, comparison was made with the control. The percentage inhibition was calculated according to the method Whipp²⁹

$$\text{Percentage inhibition} = \frac{R_1 - R_2}{R_1} \times 100\%$$

Where; R_1 is the furthest radial distance of pathogen in control tubers

R_2 is the furthest radial distance of pathogen in antagonist-incorporated tubers.

Statistical Analysis of Data

The statistical analyses used in this research work were based mostly on methods in the standard textbook, Wahua³⁰, and a computer package, Statistical Product and Service Solutions (SPSS). For each treatment, each outcome was replicated thrice using triplicate analysis. Data obtained in this study was presented as mean±standard deviation on the tables. Data were also subjected to Analysis of Variance (ANOVA). Statistical comparisons were done by using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) using the IBM (SPSS software, version 22). The level of significance was set at $P < 0.05$ (probability level).

RESULTS

A total of five fungi namely- *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Aspergillus flavus* and *Penicillium* sp were repeatedly isolated from the rot-infested tissues of the yam tuber samples. *A. niger* had the highest frequency of occurrence of 76.00%. This was followed by *F. oxysporum* with 60.00% occurrence, while *A. flavus* had the least frequency of occurrence of 36.00% (Table 1). Pathogenicity test showed that all the five fungi identified: *A. niger*, *F. oxysporum*, *R. stolonifer*, *Penicillium* spp, and *A. flavus* were all pathogenic. The most virulent amongst the fungi was *A. niger* with 100.00% rot followed by *A. flavus* with 88.00% rot while the least was *Penicillium* sp with 50.00% rot (Table 2).

Comparing the radial growth of pathogenic fungi; *A. niger*, *F. oxysporum*, *R. stolonifer*, *Penicillium* sp, and *A. flavus* was higher in the control than when paired with the Biofungicides. Results show that *Bacillus subtilis* inhibited the growth of *A. flavus* with a mean zone of inhibition of 41.33mm followed by *F. oxysporum* with a mean zone of inhibition of 38.70mm and the least was *Penicillium* sp with 17.80mm mean zone of inhibition. This showed that *Bacillus subtilis* was effective on the inhibition of

the growth of all five pathogenic test fungi and its inhibitory effect was significantly ($P<0.05$) different from the effects of the other Biofungicides. The inhibitory effects of *Trichoderma viride* showed that it reduced the growth of *R. stolonifer* with a mean zone of inhibition of 34.70mm, followed by *A. flavus* with 28.40mm mean zone of inhibition, while the least was *Penicillium* sp with 15.70mm mean zone of inhibition (Table 3). *Trichoderma harzianum* showed a reduction in the growth of *A. flavus* with 25.66mm mean zone of inhibition, followed by its inhibition of *F. oxysporum* with a mean zone of inhibition of 18.74mm, while the least inhibited pathogen was *A. niger* with 9.82mm mean zone of inhibition. The least effective of all four Biofungicides was *Pseudomonas syringae* which inhibited the growth of *A. flavus* with a mean zone of inhibition of 27.10mm, followed by *A. niger* having 13.50mm mean zone of inhibition, while the least was *R. stolonifer* with 4.50mm mean zone of inhibition (Table 3).

The *in vitro* results of the dual culture of the Biofungicides and the test pathogenic fungi showed that when the mycelium of both cultures came in contact with each other, the hyphal growth of the test pathogenic fungi was found to be inhibited by the hyphae of the four Biofungicides. The result of the dual culture also shows that the Biofungicides grew much faster than the pathogen, parasitized on the test pathogen and deprived it of absorbing the nutrients from the substrates. The test pathogenic fungi eventually died. The results of the dual culture

indicated that the Biofungicides inhibited the growth of the pathogenic test fungi at varying degrees during the incubation period. *B. subtilis* inhibited the growth of *A. flavus* significantly ($P<0.05$) with 48.67% mean percentage growth inhibition and the least inhibited was *Penicillium* sp at 13.16% (Table 4). *T. viride* inhibited the growth of *R. stolonifer* significantly ($P<0.05$) when paired together with 40.16% mean percentage growth inhibition, followed by *A. flavus* at 30.64% while the least growth inhibition was recorded by *Penicillium* sp at 12.95% when paired with *T. Viride*. *Pseudomonas syringae* inhibited the growth of *A. flavus* significantly ($P<0.05$) with 31.10% mean percentage growth inhibition when paired together, followed by *A. niger* at 12.36% and the least inhibited was *R. stolonifer* at 2.52% mean percentage growth inhibition (Table 4).

This study showed that all four Biofungicides were significantly ($P<0.05$) effective in reducing rot in the yam tubers treated *in vivo*. Reduction in rot ranged from 52.55-83.40mm in *A. niger* when paired with the four Biofungicides. In *R. stolonifer*, the rot reduction ranged from 61.02-92.60mm. In *F. oxysporum*, the rot reduction ranged from 51.60-78.59mm. In *A. flavus*, rot reduction ranged from 44.65-63.40mm. Reduction in rot ranged from 77.80-81.27mm in *Penicillium* sp when paired with the Biofungicides (Table 5). Reduction of rot indicates a decrease in the radial growth of the pathogenic test fungi. *Bacillus subtilis* reduced the rot of treated yam tubers significantly ($P<0.05$) more than the other Biofungicides.

Table 1: Frequency of Occurrence of Fungal Isolates on the Yam Tubers

Isolates	No of tuber sampled	No positive for infection	Percentage (%) occurrence
<i>Aspergillus niger</i>	25	19.00	76.00
<i>Rhizopus stolonifer</i>	25	13.00	52.00
<i>Fusarium oxysporum</i>	25	15.00	60.00
<i>Aspergillus flavus</i>	25	9.00	36.00
<i>Penicillium</i> sp	25	11.00	44.00

Table 2: Pathogenicity, Symptoms and Severity Ranking of Pathogens Causing Rot in Healthy Yam Tubers

Fungal isolate	Percentage (%) Rot after 14 days	Symptoms of Infection	Pathogenicity	Severity ranking
<i>Aspergillus niger</i>	100.00	Dry purple rot to yellowish brown firm rot with black -charcoal like margin.	+++	8.00
<i>Rhizopus stolonifer</i>	61.00	Light brown to yellowish brown soft rot	++	6.00
<i>Fusarium oxysporum</i>	75.00	Cream to brown dry rot	+++	6.00
<i>Aspergillus flavus</i>	88.00	Dark brown soft rot	+++	6.00
<i>Penicillium</i> sp	50.00	Yellowish brown soft rot	++	5.00

+++ = Highly pathogenic (> 50mm in diameter) ++ = moderately pathogenic (>10) Ranking based on 0-9 scale; 0 = least severe and 9 = most severe

Table 3: Mean Zone of Inhibition of the Test Pathogens.

Biofungicides	Mean Zone of Inhibition of the Pathogenic Test Fungi (mm)				
	<i>Aspergillus niger</i>	<i>Rhizopus stolonifer</i>	<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>	<i>Penicillium</i> sp
<i>Trichoderma harzianum</i>	9.82±0.361 ^d	15.60±0.491 ^b	18.74±0.058 ^b	25.66±0.595 ^b	10.40±0.064 ^b
<i>Trichoderma viride</i>	18.76±0.781 ^b	34.70±0.404 ^a	22.10±0.072 ^b	28.40±0.029 ^b	15.70±0.104 ^a
<i>Bacillus subtilis</i>	33.70±0.751 ^a	28.70±0.224 ^a	38.70±0.108 ^a	41.33±0.681 ^a	17.80±0.000 ^a
<i>Pseudomonas syringae</i>	13.50±0.243 ^c	4.50±0.000 ^c	11.29±0.191 ^c	27.10±0.132 ^b	7.60±0.000 ^c
Control	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000

Results are in Mean of three replicates±Standard Deviation, Mean in a Column followed by different letters differ significantly ($P<0.05$).

Table 4: Mean Percentage (%) Growth Inhibition of Pathogens (in vitro)

Biofungicides	Mean Percentage (%) Growth Inhibition of Pathogens				
	<i>Aspergillus niger</i>	<i>Rhizopus stolonifer</i>	<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>	<i>Penicillium sp</i>
<i>Trichoderma harzianum</i>	9.15±0.208 ^d	18.29±0.557 ^b	11.77±0.252 ^b	27.12±1.332 ^c	10.40±0.029 ^b
<i>Trichoderma viride</i>	19.95±0.153 ^b	40.16±0.058 ^a	19.20±0.252 ^b	30.64±0.201 ^b	12.95±0.288 ^a
<i>Bacillus subtilise</i>	42.75±0.153 ^a	35.76±0.351 ^a	38.57±1.217 ^a	48.67±0.045 ^a	13.16±0.070 ^a
<i>Pseudomonas syringae</i>	12.36±0.153 ^c	2.52±0.131 ^c	6.54±0.087 ^c	31.10±0.061 ^b	9.29±0.100 ^b

Results are in Mean of three replicates±Standard Deviation, Mean in a Column followed by different letters differ significantly (P<0.05).

Table 5: Mean Radial Growth of Pathogens (in vivo)

Biofungicides	Mean Radial Growth of Pathogens (mm)				
	<i>Aspergillus niger</i>	<i>Rhizopus stolonifer</i>	<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>	<i>Penicillium sp.</i>
<i>Trichoderma harzianum</i>	83.40±0.185 ^a	77.62±0.050 ^b	74.11±0.272 ^b	63.40±0.841 ^b	80.28±0.181 ^b
<i>Trichoderma viride</i>	73.48±0.1000 ^b	56.84±0.157 ^d	67.87±0.064 ^c	60.34±0.035 ^c	78.00±0.200 ^c
<i>Bacillus subtilise</i>	52.55±1.085 ^c	61.02±1.090 ^c	51.60±0.473 ^d	44.65±0.040 ^d	77.80±0.026 ^c
<i>Pseudomonas syringae</i>	80.45±0.243 ^a	92.60±0.191 ^a	78.50±0.087 ^a	59.94±0.061 ^c	81.27±0.100 ^b
Control	91.80±0.208 ^a	95.00±0.557 ^a	84.00±0.252 ^a	87.00±1.332 ^a	89.60±0.029 ^a

Results are in Mean of three replicates±Standard Deviation, Mean in a Column followed by different letters differ significantly (P<0.05).

DISCUSSION

The fungi consistently isolated from the rot-infested tissues of the susceptible variety of white yam tuber samples agrees with the report of Anuagasi *et al.*²⁴ who isolated similar microorganisms in association with forestry nursery seedlings. These microorganisms have been associated with post-harvest rot in yam according to reports by Amusa *et al.*¹¹; Okigbo and Odurukwe²⁶; Gwa *et al.*¹⁶; Gwa and Ekefan²⁸. These organisms infected the yam tubers during the pre-harvest stage while in the soil and also through natural openings and physical damages incurred by the tuber during post harvest operations and then manifests fully during storage^{7,11}. This is similar to the observations made in this study. Pathogenicity test revealed that fungi isolated in this study were pathogenic. This can be attributed to the potential of the pathogen to utilize the nutrients in the tubers for growth, development and other metabolic activities.

Various methods of control of post harvest rot of yam tubers such as the use of plant extracts have been reported by various workers such as Amusa *et al.*¹¹; Okigbo and Odurukwe²⁶; Anukwuorji *et al.*¹². However, the use of plant extracts has its shortcomings and is limited in its function to inhibit the growth of pathogenic microorganisms. The use of plant extracts is cumbersome to handle and the extraction medium usually interferes with its inhibitory potentials. Hence, the use of Biofungicides to control yam rots in this study because of their antagonistic properties and proven potentials to inhibit the growth of microorganism using antagonism. In this present study, Biofungicides of *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtilis* and *Pseudomonas syringae* were used to control the pathogens inducing rot in white yam and these produced a significant inhibition on the growth of post harvest yam rot fungi. This observation is in tandem with the works of Okigbo and Ikediugwu¹⁹; Okigbo and Emeka¹³; Gwa and Ekefan²⁸. It was observed that the faster rate of development of rot amongst tubers was probably related to the elevated frequency of occurrence of the pathogens.

This observation is in sync with reports of Okigbo and Ikediugwu¹⁹ who reported the prevalence of *A. niger* which maintained a higher frequency of occurrence over a more prolonged duration. The *in vitro* results of the dual culture of the Biofungicides and the test pathogenic fungi showed that when the mycelium of both cultures came in contact with each other, the hyphal growth of the test pathogenic fungi was found to be inhibited by the hyphae of the Biofungicides. The results of the dual culture indicated that the Biofungicides grew faster than the pathogens, parasitized on the test pathogen, and deprived it of absorbing the nutrients from the substrates. This observation is in tandem with Okigbo and Emeka¹³; Gwa *et al.*¹⁶; Gwa and Ekefan²⁸ who observed the same in their studies.

T. harzianum and *T. viride* inhibited the growth of all pathogenic test fungi through their ability to grow much faster than the pathogenic test fungi thus, competing efficiently for space and nutrients. This has been reported by Barbosa *et al.*³¹; Siameto *et al.*³²; Mokhtar and Aid³³ on the production of both non-volatile and volatile antibiotics by species of *Trichoderma*. These substances produced by *Trichoderma* sp served in the biological control of storage rot of yam tubers. *Bacillus subtilis* inhibited the growth of all test pathogenic fungi significantly when paired together. This observation is in tandem with Okigbo¹⁷; Okigbo³⁴ in which *B. subtilis* displaced naturally occurring mycoflora on the surface of yam tubers or caused a reduction percentage of rot in treated tubers. *Pseudomonas syringae* slightly inhibited the growth of the test pathogenic fungi when paired together. This observation is in sync with the report of Okigbo and Emeka¹³. In other studies, the saprophytic strain of the bacterium, *Pseudomonas syringae* (L-59-66) also satisfactorily controlled the difficult grape rots (*Botrytis cinerea*) and blue mold of citrus (*Penicillium citrinum*)³⁵. Arya³⁵ reported that this saprophyte has been developed into a commercial brand (Ecosuinox).

CONCLUSION

The results of this study showed that these Biofungicides;

Trichoderma harzianum, *Trichoderma viride*, *Bacillus subtilis* and *Pseudomonas syringae* have potentials to control rot in post harvest white yam tubers. Overall, *Bacillus subtilis* was recorded as the most effective in controlling the pathogenic test fungi. Thus, the use of Biofungicides as biocontrol agents is an economically viable way of suppressing post harvest rot of white yam. This can provide alternative ways in reducing rot in yams than the use of chemical fungicides.

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