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Bioefficacy of *Trichoderma* species against important fungal pathogens causing post-harvest rot in sweet potato (*Ipomoea batatas* (L.) Lam)

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ABSTRACT

Tuber rot disease constitutes a serious threat to sweet potato production worldwide, causing economic losses to farmers. This study evaluated an eco-friendly approach using four biological control agents, *Trichoderma viride, T. harzianum, T. hamatum* and *T. pseudokoningii* for the management of post-harvest tuber rot disease of sweet potato. Field surveys for infected tuber samples were conducted across four major sweet potato states in Nigeria. Rot severity in inoculated tubers was evaluated over a storage period of four months and *Trichoderma* species isolated from the rhizosphere were bioassayed for the production of metabolites to evaluate the mechanism of antibiotic production for the control of rot pathogens using Gas Chromatography-Mass Spectroscopy. A total of 24 metabolites were produced by the *Trichoderma* species and the abundance were species dependent. *Trichoderma* species significantly ($p<0.05$) inhibited rot in treated tubers at 4 months after storage. However, *T. harzianum* was most effective, reducing mycelia growth of the rot pathogens by 54.6-77.3% *in vitro* and 47.2-68.8% reduction of rot incidence *in vivo*. The efficacy of *Trichoderma* species used in this study recommends their use as alternative therapy to synthetic fungicides in the management of post-harvest rot in sweet potato.

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Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam) is mainly propagated using vines and produced by farmers for its starchy tubers. Tuber periderm varies in colour depending on the variety. The cream or white-fleshed variety is widely cultivated across various agroecological zones that are notable for sweet potato production in Nigeria and sub-Saharan Africa. It has sweet taste with high moisture content and soft skin texture. Besides the fleshy tubers, the leaves can also be harvested for food (Hu *et al.,* 2004); hence it is regarded as a vegetable crop. It is an important tuber crop in Nigeria, with an annual production of 113 million metric tonnes (FAO, 2018).

Post-harvest biodeterioration accounts for about 32.5% of annual yield losses in sweet potato production, especially in the humid tropics which lack appropriate storage and processing facilities (Agu *et al.,* 2015). The tuber has a fragile periderm and this provides entry for post-harvest pathogens, especially when damaged. The prevailing high relative humidity at periods of harvest also impacts negatively on tuber storage, hence they become vulnerable to microbial attacks, causing high yield losses. The types of rot associated with sweet potato tubers include black rot (*Ceratocystis fimbriata),* dry rot (*Aspergillus niger*), *Fusarium* root and stem rot (*Fusarium solani*), foot rot (*Plenodomus destruens*), soft rot (*Rhizopus stolonifer*) and blue mould (*Penicillium* spp.) (Rees *et al.,* 2003). Although peasant farmers are actively involved in sweet potato cultivation in Nigeria, they lack the requisite knowledge and ability to preserve harvested tubers from one growing season to the other, particularly during periods of scarcity when prices become high to earn profit. Chemical control has been the conventional method of managing sweet potato diseases in Nigeria but is hazardous to man and the environment.

Biological control is an environmentally-sound and effective means of reducing disease incidence through the use of natural enemies such as microorganisms in the ecosystem. Biological control agents seek to reduce disease incidence to very low levels, and also enhance the capability of growth promoting hormones to stimulate plant growth and ultimate yield (Pascale *et al.,* 2017). *Trichoderma* species have an outstanding tendency to produce enzymes that cause lysis of the mycelia of target pathogens, and the production of secondary metabolites or antibiotics. They are the most predominant natural fungicides used in commercial

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farming consisting of more than 1500 registered products globally (Verma *et al.,* 2007). There have been some significant achievements in the utilization of *Trichoderma harzianum* by-products in the control of a number of fungal pathogens.

Although extensive studies have been conducted on the biocontrol capability of the genus *Trichoderma* against soil-borne pathogens, there is limited information on its use in the management of post-harvest pathogens of sweet potato. Therefore, this study is significant as it evaluates the use of an environmentally-friendly strategy in the preservation of sweet potato tubers from fungal biodeterioration, which will significantly reduce the over dependence of farmers on pesticide treated potatoes that are harmful to their health and environment.

Materials and Methods

Field sampling and fungal isolation

Infected sweet potato tubers showing tuber rot disease symptoms were randomly collected from selected locations across farmers' fields based on the history of sweet potato cultivation in three Local Government Areas (LGAs) from each of four States in Nigeria. Five tuber samples were randomly collected in each of the four farms surveyed per LGA. Thus, 20 tubers showing rot symptoms were collected in each LGA, with a total of 20 ×12 LGAs = 240 tuber samples for isolation of tuber rot-causing pathogens. Necrotic lesions were cut open with a sterile scalpel to expose the infected tissues. Cut tissue sections of 1.5 mm x 1.5 mm dimension were surface-sterilized with 10% sodium hypochlorite and rinsed with sterile distilled water. Plating of samples was done on potato dextrose agar (PDA) and malt extract agar. The media were prepared by dissolving 39 g and 50 g of dehydrated powder in 1 litre of sterile distilled water, respectively. Detailed structural features of each isolate were observed using a compound microscope and identification was done following standard manual of fungi (Barnett and Hunter, 1998). Inoculum concentration was quantified and adjusted to 2 x 10^4 using the method of Dania *et al*. (2014).

Determination of rot severity and tuber weight loss in storage

The experiment was a 4 x 8 factorial in a completely randomized design with four replicates. The treatments were seven pathogens: *Lasiodiplodia theobromae, Rhizoctonia solani, Fusarium oxysporum, Macrophomina phaseolina, Rhizopus nigricans, Verticillium* sp. and *Sclerotium rolfsii* including control evaluated at four levels of months after storage (MAS), 1MAS, 2MAS, 3MAS and 4MAS. Inoculated tubers were replicated four times. Rot severity was evaluated using the same fungal pathogens that were isolated from the rotted sweet potato tubers. Fresh healthy tubers were washed with distilled water and surface-sterilized using 10% sodium hypochlorite to eliminate secondary pathogens and contaminants. Sterilized 3-mm cork borer was used to bore holes 0.5 cm deep in tubers at the proximal and distal regions and inoculated with 2 x 10^4 spores/ml. Sterile distilled water was dispensed in tubers that served as control. Inoculated tubers and control treatments were enclosed in perforated and well aerated crates and stored for four months. Weight loss and rot severity were calculated according to Sangoyomi (2004).

Isolation of Trichoderma spp. and determination of inhibitory potential

Four *Trichoderma* species: *T. harzianum*, *T. viride, T. hamatum* and *T. pseudokoningii* were isolated from sweet potato rhizosphere and cultured using the dilution plate technique (Figure 1). One gram of soil was dissolved in 100 mL sterile distilled water amended with 2% citric acid in a sterilized test tube. An aliquot of 1mL of the solution was added to 15 mL malt extract agar in each Petri dish. Isolates were further purified on *Trichoderma* selective medium (TSM) (Harman, 2006) with the following constituents (grammes per litre of distilled water): 3.0 g chloramphenicol (Sigma Chemical Co., USA), 0.25g p-dimethylaminobenzenediazo sodium sulfonate (Farbenfabrik Bayer A.G., Germany), 0.3; pentachloronitrobenzene (Olin Chemicals, USA), 0.2g rose bengal (tetrachlorotetradiodofluorescein (BDH Chemicals Ltd., England), 0.15; agar (Difco Laboratories, USA) : 0.5g $MgSO_4$ .7 H_2 O, 0.2g K_2 HPO_4, 0.9 g KC1, 0.15g NH_4 NO_3 and 1.0g glucose. *Trichoderma* isolates were identified using standard taxonomic keys (Barnett and Hunter, 1998; Samuels *et al.,* 2004). The inhibitory potential of the BCAs against the test fungi was determined using agar pairing method using three inoculation regimes: Inoculation of the pathogen a day before the biological control agents (BCA), inoculation of both the pathogen and the BCA same day, and inoculation of the pathogen a day after the BCA. Determination of pathogen inhibition by the BCAs was calculated according to Harman *et al*. (2004).

Extraction of metabolites produced by Trichoderma species

A 3-mm disk of active mycelium of each isolate was inoculated into 500 mL conical flask containing 200 mL potato dextrose broth with three replications. The culture was grown at 28 ± 2°C, 12 h alternating light and darkness on a rotary shaker Model RS-38 BL (USA) and vortexed at 150 rpm for 21 days (Banitez *et al.,* 2004). The culture filtrate was obtained by sieving the broth through a double layer of sterile cheese cloth. Extraction of metabolites was done by solvent extraction method into hexane at the ratio of 1: 1 (v/v). The hexane solvent was evaporated at 40°C from the solution using rotary evaporator, while the sample residues were re-suspended in acetone solvent. Analysis of secondary metabolites produced by the *Trichoderma* species was carried out

according to method described by Dubey *et al.* (2011). Purification of secondary metabolites was done by spotting aliquots of the solvents containing metabolites on thin layer chromatography (TLC) plates with 0.25 mm thickness of silica gel layer (Eziashi *et al*,. 2006) The plates were developed in hexane and benzene mixture in ratio of 1: 1(v/v), air-dried for 5 min and visualized by exposing them to iodine vapour for 15 min. The compounds were separated using a glass column (60 × 2cm id) containing 50 g of 60 × 120 mesh pre-activated silica gel in hexane. The column was periodically eluted with hexane-ethyl acetate, distilled on a water bath and the purity *was* read using a spectrophotometer in TLC plates.

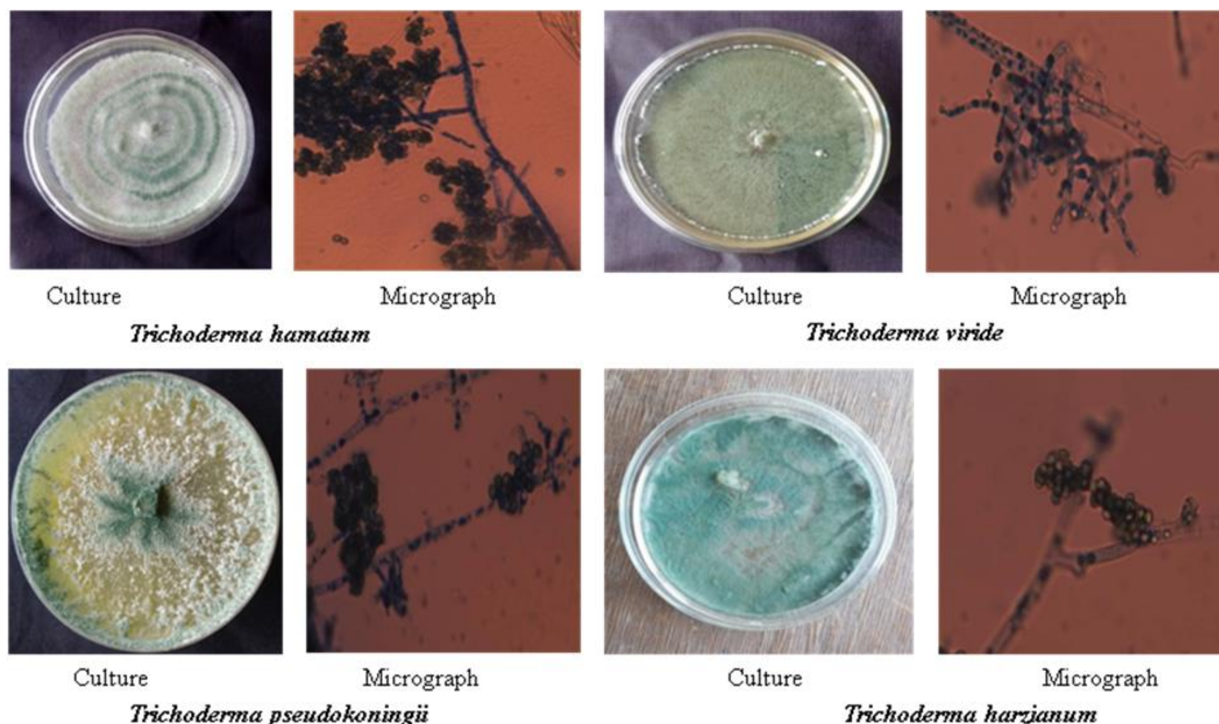

Fig. 1 Cultural and morphological features of *Trichoderma* species used in the study

Evaluation of Trichoderma species for in vivo tuber rot treatment in storage

The storage experiment was a 4 x 7 factorial laid out in completely randomized design (CRD) with three replications. The treatments were four *Trichoderma* species, *T. harzianum, T. viride, T. hamatum and T. pseudokoningii*; seven fungal pathogens, *Lasiodiplodia theobromae, Rhizoctonia solani, Fusarium oxysporum, Macrophomina phaseolina, Rhizopus nigricans, Verticillium* sp. and *Sclerotium rolfsii*. Freshly harvested healthy sweet potato tubers were washed to remove soil and debris, surface-sterilized with 10% sodium hypochlorite to remove secondary contaminants and rinsed in three changes of sterile distilled water. Healthy tubers measuring 12 cm in length and 7.5 cm girth at the middle region were used in this experiment. A 3mm-diameter cork borer was used to make 0.5 cm-deep holes and 8 cm apart at two points on the tubers. Four replicate tubers were inoculated for each organism and BCA pairing. Tubers were inoculated with 2 x 10^4 spores/mL^{-1} inoculum concentration. Tubers were inoculated using three methods: Inoculation of tubers with the pathogen a day before the BCA, inoculation of tubers with both the pathogen and the BCA same day and inoculation of tubers with the pathogen a day after the BCA. The control consisted of healthy tubers that were inoculated only with the fungal pathogens and sterile distilled water. Inoculated treatments were incubated in aerated crates at 28±2^{0}C for four months. Percent rot inhibition was calculated according to Dania *et al.* (2014).

Effect of fungal biodeterioration on proximate composition of infected tubers

Diseased sweet potato tuber samples were peeled, cut into 100 g slices and dried in a Gallenkamp oven at a temperature of 85°C for two days. The samples were allowed to cool and then pulverized into powder using a rotary blender, put in labelled McCartney bottles and refrigerated before analyses. Test samples were analyzed for moisture, crude protein, crude fibre, ash and carbohydrate using standard analytical procedures (AOAC, 1990) while employing the Kjeldahl method for crude protein determination. The lipid content was quantified following soxhlet extraction procedure

through repeated extraction for 8 hours and using aqueous hexane as organic solvent (AOAC, 2005).

Statistical analysis

Experiments were laid out in a completely randomized design with three to four replications. All data collected from the trials were subjected to analysis of variance (ANOVA) using SAS (2002) ver. 9.2 and means were separated with the Duncan's Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).

Results

Determination of rot severity and tuber weight loss in storage

Rot severity increased progressively in tubers that were inoculated with the test pathogens during the four months storage period (Table 1). *R. nigricans* which caused soft rot of inoculated tubers was the most virulent pathogen causing 66.3% rot of infected tubers at 4 months of inoculation and storage. *Verticillium* sp. was the least virulent of the test inocula with rot severity of 30.6% during the storage period. Sweet potato tubers inoculated with fungal pathogens that served as control did not exhibit rot symptoms within the first one month after storage (MAS). However, rot became apparent in these tubers as the storage progressed through the four months period. Although control tubers showed rot symptoms at successive months, rot severity was significantly lower (6.4%) relative to other treatments at 4 MAS. Weight loss which led to shrinkage of inoculated tubers became more pronounced as the storage period increased and peaked at 4 MAS.

Table 1. Effect of rot-inducing fungi on percent rot severity and weight loss of inoculated tubers in storage

	1MAS		2MAS		3MAS		4MAS	
Pathogen	Rot severity (%)	Weight loss (%)	Rot severity (%)	Weight loss (%)	Rot severity (%)	Weight Loss (%)	Rot Severity (%)	Weight Loss (%)
Lasiodiplodia theobromae	15.23±0.01^{ab}	3.01±0.05^{a}	37.44±0.5^{ab}	8.22±0.05^{a}	46.18±0.5^{b}	15.20±0.05^{ab}	60.70±1.5^{ab}	22.11±0.05^{b}
Rhizoctonia solani	18.77±1.5^{a}	3.22±0.01^{a}	38.22±0.3^{ab}	9.24±0.7^{a}	50.33±1.8^{ab}	18.01±1.5^{a}	62.18±1.2^{ab}	26.95±0.2^{ab}
Fusarium oxysporum	10.11±0.2^{b}	2.77±0.0^{a}	16.54±0.8^{c}	8.09±0.4^{a}	29.99±1.7^{cd}	13.37±0.9^{b}	43.30±0.8^{bc}	17.33±0.1^{bc}
Macrophomina phaseolina	12.88±0.4^{b}	2.61±0.0^{a}	26.33±0.1^{b}	10.01±1.1^{a}	35.71±1.5^{bc}	12.12±0.4^{b}	50.08±0.7^{b}	19.08±1.1^{b}
Rhizopus nigricans	19.45±0.6^{a}	2.91±0.5^{a}	44.21±1.2^{a}	11.77±1.5^{a}	53.07±2.2^{a}	18.42±1.3^{a}	66.33±1.8^{a}	30.05±1.5^{a}
Verticillium sp.	7.74±0.5b^{c}	2.44±0.2^{a}	18.05±0.5^{c}	6.56±0.9^{ab}	27.04±0.4^{cd}	10.75±1.1^{b}	30.61±0.3^{c}	17.08±0.1^{bc}
Sclerotium rolfsii	10.43±0.1^{b}	2.16±0.0^{a}	22.05±0.6^{bc}	5.78±0.8^{ab}	31.98±0.3^{c}	15.88±0.7^{ab}	51.88±1.5^{b}	20.55±0.3^{b}
Control	0.0±0.0^{c}	0.22±0.0a	3.1±0.0^{cd}	2.55±0.0^{b}	4.8±0.5^{d}	6.66±0.1^{bc}	6.4±0.0^{cd}	11.80±0.7c
Level of significance	**	NS	**	**	**	**	**	**
CV (%)	2.73	1.34	1.77	1.45	1.80	1.21	2.01	1.62

MAS= Months after storage; Each value represents mean ± standard error; Means with same letter along the column are not significantly different ($p<0.05$) using Duncan Multiple Range Test (DMRT); * = Significant at 5% level of probability, **= Significant at 1% level of probability; NS= Not significant.

Evaluation of Trichoderma metabolites against tuber rot pathogens

The efficacy of each *Trichoderma* species varied depending on the test pathogen and inoculation regime (Table 2). *T. harzianum* had high effectiveness on *L. theobromae, F. oxysporum* and *R. nigricans* with an inhibition range that varied from 34.8-71.4%, 42.91-73.7% and 65.8-70.4% respectively. *T. hamatum* was generally most effective against *R. solani* and *M. phaseolina* inhibiting the pathogens by 32.8-63.8% and 33.7-70.1% respectively. Similarly, *T. pseudokoningii* had the highest efficacy against two of the test pathogens, *Verticillium* sp. and *S. rolfsii* with mycelial inhibition ranging from 33.3-72.9% and 22.2-82.8% respectively. R. nigricans had significant mycelial growth when inoculated a day before *T. hamatum*. However, its radial spread decreased rapidly when inoculated same day with the BCA with complete growth inhibition when inoculated a day after relative to control. All the Trichoderma species produced a total of 24 metabolites in the Gas Chromatography-Mass Spectroscopy analysis (Table 3). However, the abundance, presence or absence of a metabolite varied the with species. *T. harzianum* and *T. pseudokoningii* produced the highest and lowest number of metabolites, respectively.

Evaluation of Trichoderma species for in vivo tuber rot treatment in storage

T. hamatum was the most effective in tubers that were either treated with the BCA after inoculation with the test pathogen or simultaneous inoculation of the pathogen and application of the BCA same day with percent rot reduction ranging from 36.7-78.4% and 47.2-68.8% respectively (Table 4). However, *T. harzianum* was most effective when the pathogens were inoculated a day after treatment with the BCA, inhibiting rot development by 54.6-77.3% across the test pathogens. Conversely, rot incidence was more pronounced in tubers without BCA application that served as control, varying significantly between 32.2% and 66.2%. *T. viride* was most effective in tubers that were inoculated with *R. solani* and treated with the four BCAs. At four months after storage, rot development was significantly reduced in the treated tubers relative to the control.

Effect of fungal biodeterioration on proximate composition of infected tubers

Sweet potato tuber samples artificially inoculated with *M. phaseolina*, *R. nigricans* and *R. solani* had the highest crude fibre content of 3.07, 3.15 and 3.01 mg/g, respectively (Table 5). However, there was no significant difference (p>0.05) between the treatments and uninoculated control. Moisture content in artificially inoculated tubers varied between 40.03% and 48.02%, while inoculated tubers that served as control had the highest moisture content of 52.08%. Ash content of inoculated tubers varied from 4.8-5.7 mg/g and did not differ significantly among the treatments. However, the ash content contained in the healthy uninoculated tubers was significantly lower than the quantity obtained from inoculated tubers. Similarly, there was no significant (p>0.05) difference in the quantity of fat and protein in the tubers inoculated with the fungal pathogens, but the control tubers differed significantly (p<0.05) with lower values for both nutrient constituents. Tubers inoculated with *L. theobromae* had the lowest carbohydrate content of 33.2 mg/g relative to the control.

Table 2. Laboratory assay of *Trichoderma* metabolites on percent mycelial reduction of tuber rot pathogens at four days after inoculation

	Trichoderma species											
	Trichoderma viride			*Trichoderma harzianum*			*Trichoderma hamatum*			*Trichoderma pseudokoningii*		
Fungus	DB	SD	DA	DB	SD	DA	DB	SD	DA	DB	SD	DA
Lasiodiplodia thebromae	40.6ab	24.5bc	47.2c	34.8bc	42.8ab	71.4a	28.5ab	22.1bc	53.3b	22.7b	26.4c	18.3cd
Rhizoctonia solani	25.2bc	48.6ab	55.6bc	40.4b	33.9b	37.0bc	32.8ab	36.4b	63.8ab	17.7bc	59.7a	54.8b
Fusarium oxysporum	65.7a	51.4ab	68.6ab	62.8ab	42.9ab	73.7a	31.4ab	57.1ab	42.9bc	22.8b	28.6c	31.4c
Macrophomina phaseolina	11.1c	25.9bc	57.7bc	23.4c	34.1b	50.0b	33.7ab	39.3b	70.1a	20.4b	44.4b	39.2bc
Rhizopus nigricans	31.5b	61.1a	73.8a	70.4a	86.4a	65.8ab	12.8bc	23.6bc	31.7c	15.5bc	35.8bc	32.9c
Verticillum sp.	33.3b	17.3c	74.1a	22.2c	36.0b	72.2a	42.7a	66.6a	71.5a	33.3a	52.8ab	72.9ab
Sclerotium rolfsii	33.9b	40.7b	62.1b	38.1b	34.3b	49.2b	18.7b	38.8b	58.7ab	22.2b	57.3a	82.8a
Control	0.0d	0.0d	0.0d	0.0d	0.0c	0.0c	0.0c	0.0c	0.0d	0.0c	0.0d	0.0d
Level of significance	**	**	**	*	**	**	**	**	**	**	*	**
CV (%)	3.51	2.77	4.03	1.97	1.41	5.01	2.66	5.03	2.55	2.10	6.33	4.09

Values are means of three replicates. Means with same letter along the column are not significantly different (p<0.05), using Duncan Multiple Range Test (DMRT); DB= Inoculation of pathogen a day before *Trichoderma* species, SD= Inoculation of pathogen and *Trichoderma* species same day, DA= Inoculation of pathogen a day after *Trichoderma* species; * = Significant at 5% level of probability, **= Significant at 1% level of probability, NS= Not significant

Table 3. Metabolite abundance (%) produced by *Trichoderma* species quantified using Gas Chromatography-Mass Spectroscopy (GC-MC)

Metabolite	*T. vride*	*T. harzianum*	*T. asperellum*	*T. pseudokoningii*
Nerodinol	0.0*	7.22	0.0	9.41
Tricho-acerenol	12.62	0.0	15.31	0.0
Acarenone	3.74	2.77	0.0	1.92
Unidentified	11.12	14.26	10.55	13.41
Verticol	8.71	6.31	4.22	0.0
Trichosane	18.22	16.71	19.2	17.44
1,3-dimethyl nenzene	0.0	6.41	4.51	7.33
2, butoxyl ethanol	2.54	3.77	0.0	0.0
2 Methyl-1- propanol	4.33	6.74	7.01	5.22
3, methoxyl-1-butanol	2.66	3.08	3.99	0.0
Unidentified	7.32	7.07	8.15	9.1
Limonene	0.0	0.0	0.0	1.02
Nephthalene	15.2	13.21	12.66	0.0
Hexadecanoic acid	3.21	0.0	3.08	3.01
Tricosane	10.31	8.75	11.2	9.33
1,3- dimethyl ethane	6.04	7.18	9.22	4.55
2,3-biolutaned	0.0	0.43	0.0	0.0
1, Methyl-2-butanol	5.21	7.03	7.92	6.22
Unidentified	14.23	0.0	13.77	14.89
2 Phenyl ethanol	6.01	5.95	7.02	6.33
1,2 dimethyl octanol	0.0	8.54	0.0	9.02
1,3 dibenzyl carboxylic acid	4.11	4.87	5.55	0.0
Alpha-cuperene	12.04	10.77	11.03	11.81
Unidentified	0.24	0.83	0.21	0.76

*=Absence of metabolite

Table 4. Effect of *Trichoderma* species on the management of rot pathogens of sweet potato stored for four months

	Trichoderma species											
	Trichoderma viride			*Trichoderma harzianum*			*Trichoderma hamatum*			*Trichoderma. pseudokoningii*		
Fungus	DB	SD	DA	DB	SD	DA	DB	SD	DA	DB	SD	DA
Lasiodiplodia theobromae	52.9b	63.2a	66.6a	60.2ab	61.2ab	54.6bc	65.1b	62.2ab	65.3ab	49.2bc	44.3bc	66.2a
Control	55.7ab	41.3c	51.2bc	47.1c	50.3b	44.2cd	58.9c	55.2b	51.3bc	47.1bc	45.7bc	50.9bc
Rhizoctonia solani	56.8ab	58.8ab	60.1ab	55.5b	51.7b	67.6b	69.3ab	67.7a	68.4a	60.3ab	58.1ab	58.8ab
Control	50.1bc	46.6bc	44.5c	62.2ab	52.1b	56.8bc	46.4e	45.3c	50.4bc	48.9bc	45.3bc	49.1bc
Fusarium oxysporum	50.5bc	47.2bc	40.6cd	50.3bc	60.4ab	71.3ab	78.4a	68.4a	70.2a	64.2a	62.8a	51.1bc
Control	39.8cd	45.3bc	37.3d	54.1b	50.7b	52.1c	45.7e	49.5bc	50.5bc	38.8cd	53.3b	40.8c
Macrophomina phaseolina	60.3a	62.1a	65.7a	55.5b	50.5b	70.2ab	53.8cd	55.9b	69.1a	54.7b	56.1ab	50.8bc
Control	37.2cd	38.7cd	34.4de	46.5c	36.5c	41.1d	44.8e	54.1b	50.2bc	35.1d	40.7c	37.9c
Rhizopus nigricans	44.2c	45.9bc	35.7de	66.6a	64.2a	76.9a	50.6d	47.2bc	65.5ab	48.2bc	57.7ab	54.1b
Control	33.3d	37.6cd	38.4d	41.2cd	32.2c	34.8de	39.7ef	33.3d	35.6c	44.1c	39.9c	41.1c
Verticillum sp.	53.1b	50.5b	55.8b	50.1bc	64.9a	77.3a	62.3bc	68.8a	65.4ab	44.5c	40.3c	66.4a
Control	43.3c	36.6cd	37.5d	40.0cd	44.3bc	33.2	36.7ef	40.1cd	31.6cd	34.4d	52.1b	35.5
Sclerotium rolfsii	55.2ab	64.4a	64.8a	49.5bc	50.9b	67.4b	48.3de	54.7b	58.8a	53.5b	60.8a	53.3b
Control	50.3bc	56.3ab	54.1b	61.1ab	60.5ab	66.2b	49.4de	55.7b	54.3b	48.8bc	57.7ab	50.2bc
Level of significance	**	**	**	**	**	**	**	**	**	**	**	**
CV (%)	6.22	5.83	7.33	1.44	2.39	6.01	2.93	3.81	4.47	4.76	2.05	3.51

Values are means of three replicates. Means with same letter along the column are not significantly different. (p<0.05), using Duncan Multiple Range Test (DMRT); DB= Inoculation of pathogen a day before *Trichoderma* species, SD= Inoculation of pathogen and *Trichoderma* species same day, DA= Inoculation of pathogen a day after *Trichoderma* species * = Significant at 5% level of probability, **= Significant at 1% level of probability, NS= Not significant.

Table 5. Effect of fungal biodeterioration on proximate composition of infected tubers

	Nutrient composition mg/g					
Fungus	Crude fibre	Moisture	Ash	Fat	Protein	Carbohydrate
Macrophomina phaseolina	3.07±0.2[a]	48.02±1.5[ab]	5.3±0.4[a]	3.8±0.1[a]	4.95±0.4[a]	43.7±1.3[ab]
Lasiodiplodia theobromae	2.94±0.5[a]	44.41±0.9[b]	5.7±0.1[a]	3.3±0.1[a]	5.8±0.7[a]	33.2±1.1[bc]
Rhizopus nigricans	3.15±0.1[a]	47.3±0.7[ab]	4.9±0.6[a]	2.9±0.5[a]	5.2±0.9[a]	37.7±0.8[bc]
Fusarium oxysporum	2.81±0.3[a]	42.05±0.8[b]	5.1±0.1[a]	3.4±0.3[a]	5.4±0.5[a]	35.5±0.3[bc]
Verticillium sp.	2.77±0.1[a]	43.31±1.1[b]	4.8±0.3[a]	4.8±0.3[a]	5.6±0.7[a]	34.8±1.2[bc]
Rhizoctonia solani	3.01±0.7[a]	41.12±1.6[b]	5.1±0.5[a]	4.1±0.1[a]	4.7±0.1[a]	40.01±0.6[b]
Sclerotium rolfsii	2.82±0.3[a]	40.03±0.6[b]	5.5±0.2[a]	3.01±0.5[a]	5.11±0.1[a]	39.1±114[b]
Control	2.72±0.1[a]	52.08±0.3[a]	3.2±0.7[b]	0.77±0.1[ab]	2.84±0.5[b]	46.4±1.5[a]
Level of significance	NS	NS	NS	NS	NS	NS
CV (%)	1.07	1.40	3.09	2.06	1.11	3.33

Each value represents mean ± standard error at P≤0.05 using Duncan Multiple Range Test; NS = Non significant

Discussion

Seven fungal genera, *Lasiodiplodia, Rhizoctonia, Fusarium, Macrophomina, Rhizopus, Verticillium* and *Sclerotium* were found to be most virulent in their rot causing ability in inoculated tubers. These results are consistent with earlier reports (Sawant *et al.,* 2012) that implicated these pathogens in post-harvest spoilage of yam. Virulence factors facilitate rot severity of pathogens by helping them to invade the host plant, cause disease and evade host defenses. These are often determined by the number of infective propagules produced by the pathogen and adaptability to prevailing environmental factors. Although there was no rot incidence in tubers inoculated with rot-inducing fungi during the first one month of storage, there was still loss of weight in the same period. This could be attributed to the fact that weight loss in tubers during storage is not exclusively due to biodeterioration. Besides pathogen invasion, physiological factors such as transpiration or evaporation of water from tuber surface, tuber respiration and sprouting, as well as damage by extremes of temperature could also influence tuber weight loss during storage.

Trichoderma species are soil-inhabiting fungi that possess inherent ability to inhibit growth of numerous other fungi that cause plant diseases, especially in the rhizophere. Results showed that the four BCAs, significantly inhibited growth of the rot-inciting fungi in the experimental trials. However, the most promising results were recorded when the pathogens were inoculated a day after the BCAs. This could be attributed to the priming effect of the *Trichoderma* species. which are notable for priming against invasive plant pathogens. Biopriming is an improved method that is adopted to improve plant growth and reduce biotic stress. This ecological approach has been reported to protect seeds against pathogens and reduces disease incidence (Jenson *et al.,* 2004). Tuber priming involves coating with beneficial soil microbes which result in rapid surface colonization. *Pythium* species have been reported to infect seeds in less than 4 hours after sowing while spore of *Trichoderma* needs nearly 12 hours to germinate (Pill

et al. 2009). This implies that biopriming against *Pythium* using *Trichoderma* will be unsuccessful if applied after infection by the pathogen. The efficacy of *Trichoderma* species in the control of plant diseases largely depends on the duration or length of time between its application and inoculation of the pathogen Pascale *et al.* (2017). Therefore, timing is very critical to achieving successful field and post-harvest application of *Trichoderma* species for the control of plant diseases.
Microscopic observation of the morphological association between the BCAs and the test pathogens in dual culture of this study showed hyphal strands of the BCA growing and coiling around those of the pathogens, which suggests hyperparasitism as a likely mechanism of control. All the BCAs evaluated in this study produced a total of 24 metabolites in the Gas Chromatography-Mass Spectroscopy analysis. This could be responsible for the distinct inhibitory zone and lysis of mycelia of test pathogens resulting from the toxic metabolites produced by the BCAs. The production of metabolites or antibiotics is an important mechanism in the control of pathogens by the BCAs. Metabolites produced by the four *Trichoderma* species, *T. viride, T. hamatum, T. pseudokoningii* that were used in this study have not been reported to be toxic to humans (Harman, 2006; Faheem *et al.,* 2010). However, strains of *Trichoderma longibrachiatum* which was not used in this study have been found to cause health issues in persons with low immunity e.g. patients diagnosed with leukemia (Samuels, 2004).

Although the mechanisms of competition, hyperparasitism, antibiosis and induction of systemic resistance are the main methods of disease control by *Trichoderma* species, a combination of these processes could likely be implicated in the control of phytopathogenic fungi by these BCAs (Samuels *et al*., 2006). *Trichoderma* species are known to produce enzymes such as chitinases, pectolytic and amylases that help them to degrade the chitin constituent contained in the cell wall of invading fungi and facilitate their entrance (Sawant, 2014). Although the *Trichoderma* species evaluated in this study significantly ($p<0.05$) reduced tuber rot incidence in the *in vivo* trials, they could not, however, completely stop the activity of the pathogens. Several authors have reported that the BCAs have the ability to minimize disease incidence considerably but absolute control is impossible because of some inherent factors. (Faheem *et al.,* 2010; Kerroun *et al.,* 2015).

Proximate analysis of the infected tubers showed an increase in the amount of protein content. This result agrees with Onifade *et al.* (2004) that reported nutritional enrichment of sweet potato through fungal fermentation process. Conversely, there was a considerable reduction in the amount of carbohydrate in decaying tubers. This may be due to the hydrolytic activity of cellulolytic enzymes secreted by the fungi which resulted in hydrolysis of complex carbohydrates to glucose, thereby serving as source of carbon and energy to the pathogens, while the reduced moisture content in infected tubers may be due to both the respiratory activity of the sweet potato tubers and the infecting pathogens.

Although every effort should be made to prevent mechanical injury to sweet potato tubers during harvesting and packing, it is almost impossible to avoid all injuries. Rot-inducing organisms, especially those that cause soft rot enter through such injuries. Bruised or crushed tissue offers favourable surface for decay to develop. Therefore, sweet potato tubers must be handled with utmost care during harvesting, transportation to storage and marketing to minimize injuries that serve as avenues for post-harvest pathogens. Although the BCAs could not completely control rot incidence due to the complexity of the pathogens involved, they will be an invaluable asset in the integrated management of the disease.

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