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% and 47.2% 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.

**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
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 x 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 of malt extract, 20 g of peptone, 5 g of sodium chloride, 1 g of yeast extract and 15 mL of sterile distilled water per litre of distilled water. After autoclaving at 121°C for 15 min, the media were seeded with 10 mL of a suspension of 10^6 spores/mL of the following fungi: *Trichoderma harzianum*, *T. viride*, *T. pseudokoningii*, *T. hamatum*, *T. asperellum*, *Fusarium oxysporum*, *Fusarium solani*, *Rhizopus nigricans*, *Macrophomina phaseolina*, *Aspergillus niger*, *Sclerotium rolfsii*, *Verticillium dahliae*, *Rhizoctonia solani*, and *Phytophthora nicotianae*. The inoculated samples were 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.

**Determination of pathogen inhibition by the BCA**

Ten percent sodium hypochlorite was used to eliminate the bacteria, and sterilized 3-mm cork borers were 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 1 mL 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 (grams per litre of distilled water): 3.0 g chloramphenicol (Sigma Chemical Co., USA), 0.25 g 1-p-dimethylaminobenzenediazoc sodium sulfonate (Farbenfabrik Bayer A.G., Germany), 0.3; pentachloronitrobenzene (Olin Chemicals, USA), 0.2 g rose bengal (tetrachlorotetradifluorescein (BDH Chemicals Ltd., England), 0.15; agar (Difco Laboratories, USA) : 0.5 g MgSO_4_7 H_2 O, 0.2 g K_2 HPO_4, 0.9 g KC1, 0.15 g NH_4 NO_3 and 1.0 g 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.
Bioefficacy of Trichoderma metabolites of sweet potato 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.

![Culture and Micrograph of Trichoderma species](image)

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

The storage experiment was a 4 × 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 × 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°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.

| Pathogen                          | 1MAS Rot (%) | 2MAS Weight (%) | 2MAS Rot (%) | 3MAS Weight (%) | 3MAS Rot (%) | 4MAS Weight (%) | 4MAS Rot (%) |
|-----------------------------------|--------------|-----------------|--------------|-----------------|--------------|-----------------|--------------|
| Lasiodiplodia theobromae         | 15.23±0.01   | 3.01±0.05       | 8.22±0.05    | 15.20±0.05      | 20.00±1.5    | 22.11±0.05      |
| Rhizoctonia solani                | 18.77±1.5    | 3.22±0.01       | 38.22±0.3    | 50.33±1.8       | 18.01±1.5    | 62.18±1.2       | 26.95±0.2    |
| Fusarium oxysporum               | 10.11±0.2    | 2.77±0.0        | 16.54±0.8    | 29.99±1.7       | 13.37±0.9    | 43.30±0.8       | 17.33±1.1    |
| Macrophomina phaseolina           | 12.88±0.4    | 2.61±0.0        | 26.33±1.1    | 35.17±1.5       | 12.12±0.4    | 12.00±0.1       | 40.83±1.6    |
| Rhizopus nigricans                | 19.45±0.6    | 2.91±0.5        | 44.21±1.2    | 53.07±2.2       | 18.42±1.3    | 46.33±1.8       | 30.05±1.5    |
| Verticillium sp.                  | 7.74±0.5     | 2.44±0.2        | 18.05±0.5    | 27.04±0.4       | 10.75±1.1    | 30.61±0.3       | 17.08±1.1    |
| Schizophyllum rolfsii             | 10.43±0.1    | 2.16±0.9        | 22.05±0.6    | 31.98±0.3       | 15.88±0.7    | 51.88±1.5       | 20.55±0.3    |
| Control                           | 0.0±0.0      | 0.22±0.0        | 3.1±0.0      | 4.8±0.5         | 6.6±0.1     | 6.4±0.0         | 11.80±0.7    |

**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. harzianum* 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.
Bioefficacy of Trichoderma metabolites of sweet potato

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 | Fungus | DB | SD | DA | DB | SD | DA | DB | SD | DA |
|---------------------|--------|----|----|----|----|----|----|----|----|----|
| Lasiodipodia thebromae | 40.6ab | 24.5bc | 47.2c | 34.8bc | 34.8bc | 28.5ab | 22.1bc | 53.3b | 22.7b | 26.4c | 18.3cd |
| Rhizoctonia solani | 25.2bc | 48.6ab | 55.6bc | 40.4bc | 33.9bc | 37.0bc | 32.8ab | 36.4bc | 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 | 23.6c | 31.7c | 15.5bc | 35.8bc | 32.9c |
| Verticillium 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 | 0.0d | 0.0c | 0.0d | 0.0d | 0.0d |

Level of significance: ** = Significant at 5% level of probability, *** = Significant at 1% level of probability, NS = Not significant

| CV (%) | 3.51 | 2.77 | 4.03 | 1.97 | 1.41 | 5.01 | 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. vireae | T. harzianum | T. asperellum | T. pseudokoningii |
|------------|-----------|-------------|---------------|------------------|
| Nerodinol  | 0.0*      | 7.22        | 0.0           | 9.41             |
| Tricho-acenol | 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-dimethylnenzene | 0.0    | 6.41        | 4.51          | 7.33             |
| 2, butoxyl ethanol | 2.54   | 3.77        | 0.0           | 0.0              |
| Methyl-1-propanol  | 4.33    | 6.74        | 7.01          | 5.22             |
| 3, methyl-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             |
| Naphthalene | 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-biolutan | 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 viride | Trichoderma harzianum | Trichoderma hamatum | Trichoderma, pseudokoningii |
|-------------------|-----------------------|---------------------|-----------------------------|
| **Fungus**        | **DB** | **SD** | **DA** | **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            | 55.7a | 41.3c | 51.2bc | 47.1c | 50.3b | 44.2cd | 58.9c | 55.2b | 51.3bc | 47.1c | 45.7bc | 50.9bc |
| Rhizoctonia solani | 56.8a | 58.8a | 60.1ab | 55.5b | 51.7b | 67.6b | 69.5ab | 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 | 34.4d | 46.5c | 36.5c | 41.1d | 44.8e | 54.1b | 50.2bc | 35.1d | 40.7c | 37.9c |
| Rhizopus nigricans | 44.2c | 45.9bc | 35.7d | 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 | 32.3e | 34.8e | 39.7ef | 33.3d | 35.6c | 44.1c | 39.9e | 41.1c |
| Verticillium 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 | 33.2e | 36.7ef | 40.1cd | 31.6cd | 34.4d | 52.1b | 35.5 |
| Sclerotium rolfsii  | 55.2a | 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 | 66.2bc | 49.4de | 55.7b | 54.3b | 48.8bc | 57.7ab | 50.2bc |

Level of significance

| CV (%) | ** | ** | ** | ** | ** | ** | ** |
|---------|----|----|----|----|----|----|----|
| 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 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

| **Fungus** | **Crude fibre** | **Moisture** | **Nutrient composition mg/g** | **Ash** | **Fat** | **Protein** | **Carbohydrate** |
|------------|-----------------|-------------|-------------------------------|--------|--------|------------|-----------------|
| Macrophomina phaseolina | 3.07±0.2a | 48.02±1.5ab | 5.34±0.4a | 3.8±0.1a | 4.95±0.4a | 43.7±1.3ab |
| Lasiodiplodia theobromae | 2.94±0.5ab | 44.4±1.9ab | 5.7±0.1a | 3.3±0.1a | 5.8±0.7a | 33.2±1.1bc |
| Rhizopus nigricans | 3.15±0.1a | 47.3±0.2ab | 4.9±0.6a | 2.9±0.5a | 5.2±0.9a | 37.7±0.8bc |
| Fusarium oxysporum | 2.81±0.3a | 42.05±0.8ab | 5.1±0.1a | 3.4±0.3a | 5.4±0.5a | 35.5±0.3bc |
| Verticillium sp. | 2.77±0.1a | 43.3±1.1ab | 4.8±0.3a | 4.8±0.3a | 5.6±0.7a | 34.3±1.2bc |
| Rhizoctonia solani | 3.01±0.7a | 41.1±1.6ab | 5.1±0.5a | 4.1±0.1a | 4.7±0.1a | 40.01±0.6bc |
| Sclerotium rolfsii | 2.82±0.3a | 40.03±0.6ab | 5.5±0.2a | 3.01±0.5a | 5.1±0.1a | 39.1±114bc |
| Control | 2.72±0.1a | 52.08±0.3ab | 3.2±0.7b | 0.7±0.1ab | 2.8±0.5b | 46.4±1.5a |

Level of significance

| **CV (%)** | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** |
|------------|--------|--------|--------|--------|--------|--------|
| 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 rhizosphere. 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...
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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 cellulosytic 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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