

CONTROL OF POSTHARVEST ROTS OF BANANA FRUITS BY CONIDIA AND CULTURE FILTRATES OF *TRICHODERMA ASPERELLUM*

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Received: March 6, 2009

Accepted: July 30, 2009

Abstract: Banana fruits are highly perishable and prone to microbial infection that cause significant damage. Fungicides and pesticides that are used to control this infection are toxic to man and animals, hence there is the need for environmentally friendly control measures of fruit rot pathogens. Simultaneous inoculation of fruits with *Trichoderma* species and rot pathogens resulted in rot on the fingers, but rot produced by *T. asperellum* NG-T161 alone or in combination with the pathogens was reduced, compared to rot produced by the pathogens alone. Treatment of fruits with conidia and culture filtrates of *T. asperellum* NG-T161 for 30 min prior to inoculation with the pathogens provided a better control than their simultaneous application. Only *Trichoderma* species were recovered on plated portions of rotted tissues from inoculations with the pathogens and the antagonists on the fruits. At 50% (v/v) the filtrates inhibited the mycelial growth of *Fusarium oxysporum* and *Colletotrichum musae* by 49.7 and 60.3% respectively but *Lasiodiplodia theobromae* was not inhibited. *T. asperellum* strains were found to be mycoparasitic on banana fruit rot pathogens. Conidia and culture filtrates of *T. asperellum* NG-T161 controlled the rot on banana fruits. It could be developed into a biopesticide for the control of postharvest banana fruit rot pathogens.

Key words: banana, *Trichoderma asperellum*, conidia, culture filtrates

INTRODUCTION

Commercial control of crown rot of banana consists of postharvest treatment with thiabendazole and/or imazalil (Finlay and Brown 1993). Both are used in conjunction for crown rot control, because neither is sufficiently effective as a sole postharvest treatment (Krauss *et al.* 1998). Fungicide resistance in *Colletotrichum musae*, especially against thiabendazole, was reported (Griffie 1976; Johanson and Blazquez 1992; de Lapeyre de Bellaire and Dubois 1997). Resistance to benzimidazole fungicides of fungal pathogens of banana fruits is common and may have been encouraged by the use of fungicides with the same mode of action for control of foliar diseases in the plantations (Slabaugh and Grove 1982; Johanson and Blazquez 1992). The intensive and continuous use of benomyl resulted in the development of resistant strains of crown rot pathogens in banana fruits (Slabaugh and Grove 1982).

Trichoderma species have a potential as biological control agents against seed and root rot pathogens and for the management of postharvest diseases (Papavivas 1985; Ghisalberti and Sivasithamparam 1991; Mortuza and Ilag 1999; Okigbo and Ikediugwu 2000). Some strains of *Trichoderma* had also been identified as potential biological control agents of plant pathogenic fungi on many crops

including strawberries (*Fragaria vesca* T Jun), cucumbers (*Cucumis sativus* L.), tomatoes (*Lycopersicon esculentum* Mill.), radishes (*Raphanus sativus* L.), sugar beets (*Beta vulgaris* L.) and cotton (*Gossypium hirsutum* L. Syn.) (Ozbay and Newman 2004).

Trichoderma harzianum Rifai, *T. viride* Pers.ex Gray, *T. polysporium* (Link ex Pers.), *T. longibrachiatum* Rifai, *T. koningii* Oudem. are important control agents of plant pathogens. They were found to be effective in controlling a large number of plant pathogenic fungi such as *Sclerotium rolfsii*, *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum*, *Phytophthora* spp., *Phythium* sp., *Fusarium* spp., *Protomyces phaseoli* and *Botrytis cinerea* Micheli ex Persoon. These bioagents were used for the control of foliar, soil-borne, and postharvest diseases in various crops in the field, in commercial greenhouses and storage depots (Papavivas 1985; Elad 1994; Adejumo *et al.* 1999; Sobowale *et al.* 2005).

Mortuza and Ilag (1999) used conidia and culture filtrates of *Trichoderma* species to reduce rotting on bananas artificially inoculated with *Lasiodiplodia theobromae* (Pat). Griff and Maubi. Tronsmo and Dennis (1977) were able to protect strawberry fruits against storage rot caused by *Botrytis cinerea* and *Mucor mucedo* by spraying strawberry

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plants in the field during early flowering, with aqueous suspensions of conidia of *T. viride* and *T. polysporum*.

Kesiang *et al.* (2002) found the efficacy of the control of apple ring rot caused by *Botryosphaeria beregeriana* f. sp. *pericola* with *Trichoderma* to be similar to that of a chemical control. Odebode and Sobowale (2001) found out that species of *Trichoderma* have antagonistic activity against *Aspergillus niger*, *Rhizopus nigricans*, *Penicillium citrium* and *Oidium* sp., the postharvest rot pathogens of pepper fruit (*Capsicum annuum*). The mode of antagonism was reported to be by nutrient competition and mycoparasitism in some cases. Successful antagonism also occurred when the antagonists were inoculated several hours before inoculation with the pathogens.

The success of *Trichoderma* strains as biological control agents (BCAs) is due to their high reproductive capacity, ability to survive under highly unfavourable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi, and efficiency in promoting plant growth and defence mechanisms (Benitez *et al.* 2004).

This research aimed at exploring the possibility of using conidia and culture filtrates of *T. asperellum* in biological control of *Colletotrichum musae*, *Fusarium oxysporum* and *Lasiodiplodia theobromae*, postharvest rot pathogens of banana fruits.

MATERIALS AND METHODS

The *T. asperellum* strains used in this study were isolated from banana farm soils in South Western Nigeria and were identified at the Plant Science Institute, Systematic Botany and Mycology Laboratory, USDA Agricultural Service, Baltimore Avenue, Beltsville MD 20705-2350. The banana pathogens *C. musae*, *F. oxysporum* and *L. theobromae* used were previously isolated from banana and plantain fruits (Adebesin *et al.* 2005).

Determination of the effect of culture filtrates of *Trichoderma* species on fruit rot pathogens

The antifungal effects of culture filtrates of *T. asperellum* strains were tested against *F. oxysporum*, *C. musae* and *L. theobromae* isolated from banana and plantain fruit tissues. The method described by Shillingford (1976) was adopted. Plates were prepared by incorporating sterile culture filtrates at 50% v/v of *Trichoderma* species (sterilized by membrane filtration using 0.02 µm Millipore filters) or with sterile water (control) in melted PDA. In the case of the 50% culture filtrates, PDA was prepared with additional 5 g/l agar in order for the medium to solidify after mixing the filtrate and medium.

When agar cooled and solidified, each plate was inoculated at a central point with 5 mm disc of mycelium cut out from the edge of 5-day old colonies of *C. musae*, *F. oxysporum* and *L. theobromae*. Colony diameters were measured for three replicate plates per treatment after 5 days incubation at 28°C and the radial growth reduction was determined and compared to the radial growth of the control. Control plates were PDA plates without culture filtrates.

In vivo inoculation of *Trichoderma* species and rot pathogens on fruit

The banana and plantain fruits without bruises or spots were washed in sterile distilled water and allowed to drain dry. The fingers were then soaked in 10% sodium hypochlorite for 3 min and allowed to dry in the laminar flow hood. Each finger was inoculated with 0.1 ml spore suspension ($\times 10^6$ spores/ml of each of *C. musae* and *F. oxysporum* and 10^4 spores/ml of *L. theobromae*) and then with 0.1 ml of spore suspension ($\times 10^6$ spores/ml) of each of the *Trichoderma* species at a central point on the finger simultaneously. Each treatment was replicated thrice with four fingers per replicate. Control was set up with inoculation of fruits with the pathogens alone, the antagonist alone, and with sterile water. The inoculated fruits were stored in a humid chamber in the laboratory and fingers were observed for rot development. The fruits were sliced along the point of inoculation and the extent of rot determined after 7 days of incubation. Isolations were made from rotted portions to determine the effect of the antagonists on the pathogens.

Effect of culture filtrates on disease development

The method adopted to determine the effect of the culture filtrates of *Trichoderma asperellum* was similar to that used by Mortuza and Ilag (1999). Fresh green banana fruits were washed with water to remove adhering debris and surface-disinfected by immersion in 10% commercial bleach (Chlorox) for 5 min, air dried, and placed in plastic trays. Each fruit was wounded by piercing with scalpels (about 5 mm wide and 3 mm deep). Immediately after wounding, 20 µl of culture filtrate of *T. asperellum* NG T-161 was pipetted into each wound. After 30 min, the antagonist was challenged with a 20 µl spore suspension (10^6 conidia/ml for *F. oxysporum* and *C. musae* and 10^4 propagules/ml for *L. theobromae*). Inoculated fruits were incubated at room temperature ($28 \pm 2^\circ\text{C}$). Disease development was monitored after 5 days. Control fruits were treated only with the pathogens. Inoculation was done in duplicate with 4 fingers of fruits per treatment.

Biocontrol ability of antagonists on fruit

The method adopted to determine the biocontrol ability of *Trichoderma asperellum* on fruits was similar to that used by Mortuza and Ilag (1999). Banana fruits (Paranta) were surface-disinfected and wounded as before. A 20 µl spore suspension of the test antagonist was pipetted into each wound. The suspension was allowed to dry and then a 20 µl spore suspension of the pathogen was applied. The treatments consisted of concentrations of *T. asperellum* NG T-161 adjusted to 10^6 , 10^7 , and 10^8 conidia/ml. The inoculum level of the pathogens was 10^6 conidia/ml for *F. oxysporum* and *C. musae* and 10^4 propagules/ml for *L. theobromae*. Control fruits were treated only with the pathogens. Inoculated fruits were placed in plastic trays and incubated at room temperature ($28 \pm 2^\circ\text{C}$). Rot diameter at the wound site was measured after 5 days. Inoculation was done in duplicate with 4 fruits per treatment.

RESULTS

Effect of culture filtrates obtained from potato dextrose broth

When 50% cell free culture filtrates of the antagonists were incorporated into PDA and the pathogens were inoculated centrally, there was a reduction in the mycelial growth of *F. oxysporum* and *C. musae*. The culture filtrates had no effect on mycelial growth of *L. theobromae*, as both control and test plates had filled the Petri dishes within 4 days. Table 1 shows the effect of 50% v/v cell free culture filtrates of the *T. asperellum* strains on the three rot pathogens of fruits of *Musa* spp.

Trichoderma asperellum NG-T161 reduced the mycelial growth of *F. oxysporum* to 49.7% and that of *C. musae* to 60.3%. The culture filtrates of *T. asperellum* NG-T158 had the least effect on mycelial growth of *F. oxysporum* (8.6%) and *C. musae* (6.2%).

In vivo inoculation of *Trichoderma* species with rot pathogens on fruits

When the *Trichoderma* species were inoculated together with either *Colletotrichum musae* or *F. oxysporum* on fruit surfaces, the rot that developed on fruits had

a larger diameter than rot produced when either pathogen was inoculated alone on fruits (Tables 2, 3). However, when the fruits were inoculated with *Lasiodiplodia theobromae* and the antagonists, observed rot was not as severe as when the fingers were inoculated with *L. theobromae* alone (Table 4). No significant rot reduction was observed when the antagonists were inoculated together with the pathogens on fingers except for the antagonists with *L. theobromae*. When the banana fruits were inoculated with *T. asperellum* NG-T161 alone or *T. asperellum* NG-T161 with each of the pathogens, *C. musae*, *F. oxysporum* and *L. theobromae*, diameter of a lesion and rot percentage of the transverse section of fruit was less than when the fruits

Table 1. Effect of cell-free culture filtrates of *Trichoderma* species at 50% v/v on fruit rot pathogens of *Musa* spp.

| Trichoderma culture filtrates | Radial growth reduction [%] | | |
|-------------------------------|-----------------------------|-----------------|----------------------|
| | <i>F. oxysporum</i> | <i>C. musae</i> | <i>L. theobromae</i> |
| <i>T. asperellum</i> NG-T159 | 12.1 c | 14.5 c | 0.0 |
| <i>T. asperellum</i> NG-T161 | 49.7 a | 60.3 a | 0.0 |
| <i>T. asperellum</i> NG-T158 | 8.6 d | 6.2 d | 0.0 |
| <i>T. asperellum</i> NG-T166 | 26.3 b | 54.2 b | 0.0 |
| Means | 24.2 | 33.8 | 0.0 |
| Pr > F | < .0001 | < .0001 | . |

Means followed by the same letter within a column are not significantly different at $p \leq 0.05$

Culture filtrates are from *T. asperellum* strains grown on potato dextrose broth for 7 days on rotary shaker at 150 rpm at 28±2°C

Table 2. Length of rot lesions and extent of rot on transverse section of cavendish and *Paranta* fruits 7 days after inoculation with *F. oxysporum* and *Trichoderma* spp.

| Treatment | Length of rot lesion [mm] | | % rot of TS of fruit | |
|--|---------------------------|----------|----------------------|-----------|
| | cavendish | Paranta | cavendish | Paranta |
| Control (sterile water) | 4.4 i | 4.7 i | 0.0 f | 0.0 h |
| <i>F. oxysporum</i> alone | 7.8 h | 7.8 ghi | 48 d | 39.7 f |
| <i>T. asperellum</i> NG-T159 alone | 16.5 c | 30.8 a | 75.2 ab | 71.3 ab |
| <i>T. asperellum</i> NG-T161 alone | 6.5 h | 7.3 h i | 20.2 e | 26.7 g |
| <i>T. asperellum</i> NG-T158 alone | 28.5 a | 12.5 fgh | 79.2 a | 65.7 bcd |
| <i>T. asperellum</i> NG-T166 alone | 11.4 fg | 26.8 ab | 68.7 ab | 79.3 a |
| <i>T. asperellum</i> NG-T163 alone | 11.6 fg | 25.9 abc | 66.2 bc | 65.3 bcde |
| <i>T. asperellum</i> NG-T160 alone | 19.4 b | 21.7 bcd | 66.7 ab | 67.9 bc |
| <i>F. oxysporum</i> + <i>T. asperellum</i> NG-T159 | 12.7 ef | 17.1 def | 69.7 ab | 55.2 e |
| <i>F. oxysporum</i> + <i>T. asperellum</i> NG-T161 | 6.3 hi | 14.6 ef | 41.2 d | 56.5 de |
| <i>F. oxysporum</i> + <i>T. asperellum</i> NG-T158 | 14.8 cd | 21.1 cd | 67.8 ab | 69.4 abc |
| <i>F. oxysporum</i> + <i>T. asperellum</i> NG-T166 | 7.8 h | 18.2 cd | 53.5 cd | 60.3 cde |
| <i>F. oxysporum</i> + <i>T. asperellum</i> NG-T163 | 10.6 g | 15.5 ef | 65.9 bc | 55.7 de |
| <i>F. oxysporum</i> + <i>T. asperellum</i> NG-T160 | 14.4 de | 12.8 efg | 66.3 b | 55.6 de |
| Means | 12.3 | 16.9 | 56.3 | 54.9 |
| Pr > F | < .0001 | < .0001 | < .0001 | < .0001 |

Means followed by the same letter(s) within a column are not significantly different at $p \leq 0.05$

Table 3. Length of rot lesions and extent of rot on transverse section of cavendish and *Paranta* fruits 7 days after inoculation with *C. musae* and *Trichoderma* spp.

| Treatment | Length of rot lesion (mm) | | % Rot of TS of fruit | |
|--|---------------------------|---------|----------------------|----------|
| | cavendish | Paranta | cavendish | Paranta |
| Control (sterile water) | 4.4 f | 4.7 f | 0.0 e | 0.0 h |
| <i>C. musae</i> alone | 5.2 fe | 10.3 fe | 44.0 c | 48.8 f |
| <i>T. asperellum</i> NG-T159 alone | 16.5 c | 30.8 a | 75.2 a | 71.3 ab |
| <i>T. asperellum</i> NG-T161 alone | 6.5 e | 7.3 fe | 20.2 d | 26.7 g |
| <i>T. asperellum</i> NG-T158 alone | 28.5 a | 12.3 de | 79.2 a | 65.7 bcd |
| <i>T. asperellum</i> NG-T166 alone | 11.4 d | 26.8 ab | 68.7 ab | 79.3 a |
| <i>T. asperellum</i> NG-T163 alone | 11.6 d | 25.9 ab | 66.2 ab | 65.3 bcd |
| <i>T. asperellum</i> NG-T160 alone | 19.4 b | 21.7 bc | 66.7 ab | 67.9 bc |
| <i>C. musae</i> + <i>T. asperellum</i> NG-T159 | 16.3 c | 18.5 dc | 58.0 b | 65.3 bcd |
| <i>C. musae</i> + <i>T. asperellum</i> NG-T161 | 5.8 fe | 21.0 bc | 44.0 c | 68.4 bc |
| <i>C. musae</i> + <i>T. asperellum</i> NG-T158 | 12.2 d | 20.8 bc | 68.7 ab | 58.9 cde |
| <i>C. musae</i> + <i>T. asperellum</i> NG-T166 | 16.8 c | 12.8 de | 55.7 bc | 54.2 ef |
| <i>C. musae</i> + <i>T. asperellum</i> NG-T163 | 17.1 c | 23.8 bc | 57.1 bc | 56.2 def |
| <i>C. musae</i> + <i>T. asperellum</i> NG-T160 | 16.5 c | 23.6 bc | 57.3 bc | 70.5 ab |
| Means | 13.4 | 18.6 | 54.4 | 57.1 |
| Pr > F | < .0001 | < .0001 | < .0001 | < .0001 |

Means followed by the same letter(s) within a column are not significantly different at $p \leq 0.05$

Table 4. Length of rot lesions and extent of rot on transverse section of cavendish and *Paranta* fruits 7 days after inoculation with *L. theobromae* and *Trichoderma* spp.

| Treatment | Length of rot lesion [mm] | | % rot of TS of fruit | |
|---|---------------------------|----------|----------------------|---------|
| | cavendish | Paranta | cavendish | Paranta |
| Control (sterile water) | 4.4 h | 4.7 f | 0.0 f | 0.0 f |
| <i>L. theobromae</i> alone | 98.3 a | 126.2 a | 100.0 a | 98.7 a |
| <i>T. asperellum</i> NG-T159 alone | 16.5 efg | 30.8 d | 75.3 cd | 71.3 cd |
| <i>T. asperellum</i> NG-T161 alone | 6.5 gh | 7.3 ef | 20.2 e | 26.7 e |
| <i>T. asperellum</i> NG-T158 alone | 28.5 cd | 12.5 def | 79.2 bcd | 65.7 d |
| <i>T. asperellum</i> NG-T166 alone | 11.4 fgh | 26.8 d | 68.7 cd | 79.3 c |
| <i>T. asperellum</i> NG-T163 alone | 11.6 fgh | 25.9 de | 66.2 d | 65.3 d |
| <i>T. asperellum</i> NG-T160 alone | 19.4 edf | 21.7 def | 66.7 d | 67.9 d |
| <i>L. theobromae</i> + <i>T. asperellum</i> NG-T159 | 37.7 bc | 50.8 c | 85.2 abc | 67.8 d |
| <i>L. theobromae</i> + <i>T. asperellum</i> NG-T161 | 41.9 b | 65.8 bc | 78.6 bcd | 63.7 d |
| <i>L. theobromae</i> + <i>T. asperellum</i> NG-T158 | ND* | 58.2 c | ND | 92.6 ab |
| <i>L. theobromae</i> + <i>T. asperellum</i> NG-T166 | 25.3 ed | 31.2 d | 94.4 ab | 82.1 bc |
| <i>L. theobromae</i> + <i>T. asperellum</i> NG-T163 | ND | 78.3 b | ND | 91.7 ab |
| <i>L. theobromae</i> + <i>T. asperellum</i> NG-T160 | ND | 26.9 d | ND | 80.2 c |
| Means | 27.4 | 40.5 | 66.8 | 68.1 |
| Pr > F | < .0001 | < .0001 | < .0001 | < .0001 |

Means followed by the same letter(s) within a column are not significantly different at $p \leq 0.05$

*ND – not determined

were inoculated with the pathogens alone. The depth of rot across the fingers also varied with each antagonist and pathogen (Tables 2, 3, 4). The antagonists (*Trichoderma* spp.) only were isolated from portions of fruits inoculated with both the pathogens and antagonists.

Effect of culture filtrates and conidia of *T. asperellum* NG-T161 on infection with the pathogen of *Paranta* incubated for 4 days after inoculation

The effect of treating *Paranta* fruits with culture filtrates and conidia of *T. asperellum* NG-T161 are shown in table 5. When cell-free culture filtrates of *T. asperellum* NG-T161 were applied 30 minutes before the introduction of *F. oxysporum*, *C. musae* and *L. theobromae* into the fruits, the rot produced on the skin of the fruits was reduced by

100, 85.5 and 94.1% respectively, when compared to the fruits inoculated with each of the pathogens alone. Concentration of conidia of *T. asperellum* of 10⁶ conidia/ml, when applied unto the fruits 30 minutes before the introduction of each pathogen, reduced rot produced on the fruits by 89.6, 75.5 and 100% for *F. oxysporum*, *C. musae* and *L. theobromae*, respectively. None of the three pathogens produced rot in *Paranta* fruits when the fruits were treated with 10⁷ and 10⁸ conidia/ml of *T. asperellum* NG-T161.

None of the treatments with either the cell-free culture filtrates or conidia of *T. asperellum* NG-T161 produced any internal lesions on the fruit pulp as compared to the control treatments which were inoculated with the pathogens alone.

Table 5. Effect of culture filtrates and conidia of *T. asperellum* NG-T161 on infection of *Paranta* fruits by *F. oxysporum*, *C. musae* and *L. theobromae* after 4-days of infection

| Treatment | Length of rot lesion on skin [mm] * | % rot reduction on skin | Rot lesion diameter of TS [mm] |
|---|-------------------------------------|-------------------------|--------------------------------|
| <i>F. oxysporum</i> alone | 16.0 b | – | 12.0 b |
| <i>C. musae</i> alone | 10.3 c | – | 9.0 c |
| <i>L. theobromae</i> alone | 33.5 a | – | 20.5 a |
| <i>T. asperellum</i> culture filtrate + <i>F. oxysporum</i> | 0.0 e | 100 a | 0.0 a |
| <i>T. asperellum</i> culture filtrate + <i>C. musae</i> | 1.8 de | 85.5 bc | 0.0 a |
| <i>T. asperellum</i> culture filtrate + <i>L. theobromae</i> | 2.0 de | 94.1 ab | 0.0 a |
| <i>T. asperellum</i> conidia (10 ⁶ /ml) + <i>F. oxysporum</i> | 1.5 de | 89.6 ab | 0.0 a |
| <i>T. asperellum</i> conidia (10 ⁶ /ml) + <i>C. musae</i> | 2.5 d | 75.5 c | 0.0 a |
| <i>T. asperellum</i> conidia (10 ⁶ /ml) + <i>L. theobromae</i> | 0.0 e | 100 a | 0.0 a |
| <i>T. asperellum</i> conidia (10 ⁷ /ml) + <i>F. oxysporum</i> | 0.0 e | 100 a | 0.0 a |
| <i>T. asperellum</i> conidia (10 ⁷ /ml) + <i>C. musae</i> | 0.0 e | 100 a | 0.0 a |
| <i>T. asperellum</i> conidia (10 ⁷ /ml) + <i>L. theobromae</i> | 0.0 e | 100 a | 0.0 a |
| <i>T. asperellum</i> conidia (10 ⁸ /ml) + <i>F. oxysporum</i> | 0.0 e | 100 a | 0.0 a |
| <i>T. asperellum</i> conidia (10 ⁸ /ml) + <i>C. musae</i> | 0.0 e | 100 a | 0.0 a |
| <i>T. asperellum</i> conidia (10 ⁸ /ml) + <i>L. theobromae</i> | 0.0 e | 100 a | 0.0 a |
| Mean | 4.5 | 95.4 | 2.8 |
| Pr > F | < .0001 | 0.0083 | < .0001 |

Means followed by the same letter within a column are not significantly different at p ≤ 0.05

DISCUSSION

Trichoderma spp. were the only microorganisms isolated from rotted portions of the fruits when inoculated with the test antagonists and the pathogens together. The test pathogens (*F. oxysporum*, *C. musae* and *L. theobromae*) were not isolated. The recovery of *Trichoderma* spp. alone is a clear indication of suppression of the pathogens by *Trichoderma* species. As well Howell (2003) reported that, when root segments treated with *T. virens*, taken from the soil heavily infested with propagules of *Macrophomina phaseolina* (pathogen causing charcoal rot), were plated on agar medium at room temperature, only *T. virens* grew from the treated cotton roots.

There was a reduction of rot lesion diameter when fruits were inoculated with *L. theobromae* and the antagonists as compared to when fruits were inoculated with *L. theobromae* alone. This is an indication that the tested

Trichoderma species utilized the nutrients of both the pathogens and the fruits in getting established. *Trichoderma asperellum* NG-T158, NG-T159, NG-T166, NG-T163 and NG-T160 produced rots that were larger than those produced by each of *C. musae* and *F. oxysporum*, when individually inoculated into the fruits. The *Trichoderma* spp. could therefore be pathogenic on wounded fruits. Mortuza and Ilag (1999) reported that *T. harzianum* and *T. viride* significantly reduced *L. theobromae* infection (rot diameter) in banana fruits, but were not able to completely control fruit rot caused by *L. theobromae*. In this study, there was a reduction in length of rots produced when the fruits were simultaneously artificially inoculated with *L. theobromae* and each of the antagonists (*Trichoderma* spp.). The highest reduction of rot lesion length was obtained with *T. asperellum* NG-T161.

Roberts (1990) reported that treating of wounds with cell-free culture filtrates of *Cryptococcus laurentii* was not effective in preventing decay caused by *Botrytis cinera* on apples. The treatment of artificially induced wounds of banana fruits with cell-free culture filtrates of *T. asperellum* NG-T161 before the pathogens greatly reduced rot on the fruit surface. Mortuza and Ilag (1999) found out that *T. viride* exhibited biocontrol activity more strongly when applied prior to the pathogens and was not effective against infection already established on the fruits. This indicates that the nature of biocontrol activity is protective. The suppression of pathogens by culture filtrates on the fruits is an indication that the antifungal property of the *T. asperellum* strains may depend on metabolites produced by antagonists. The application of increasing concentrations of conidia of *T. asperellum* prior to infection of fruits with pathogens prevented rotting of fruits by pathogens.

Cell-free culture filtrates from *T. asperellum* strains grown on potato dextrose broth at a concentration of 50% v/v were able to cause a reduction in the mycelial growth of both *F. oxysporum* and *C. musae*. Hence these filtrates were inhibitory to both pathogens but they did not reduce the mycelial growth of *L. theobromae*. In this study, cell-free culture filtrates produced by *T. asperellum* NG-T161 was found to be the most effective against *F. oxysporum* and *C. musae*. Bastos (1988) found that culture filtrates obtained from *T. viride* grown in potato dextrose broth did not have an inhibitory effect on the mycelial growth of *C. pernicioso* nor on formation of basidiocarps on dead brooms. However, Odebode (2006) reported that undiluted culture filtrates of *T. harzianum* and *T. pseudokoningii* completely inhibited conidia of *Macrophomina phaseolina*, *F. solani*, *Aspergillus niger* and *Alternaria* sp. Jackson *et al.* (1991) reported that *Gliocladium virens*, *T. pseudokoningii* and *T. virens* produced antifungal metabolites which were effective in reducing the radial growth of *Rhizoctonia solani* and *Botrytis cinerea* as well as *Sclerotium cepivorum*. They also found that metabolites produced by *G. virens* were very active against all three pathogens while the metabolites produced by *T. pseudokoningii* and *T. viride* were less active.

The inability of culture filtrates to inhibit or retard the growth of *L. theobromae* indicates that it might be better to use methods which allow direct confrontation between the antagonists and the pathogens, as observed when the pathogens and the antagonists were inoculated together on fruits.

Since most of the organisms responsible for postharvest rots of the fruits of *Musa* species, especially *Colletotrichum musae*, start the infection preharvest, it is essential to develop measures that will reduce the incidence of postharvest rot by exploiting the use of *T. asperellum* conidia and culture filtrates as both soil and fruit treatments pre- and postharvest. Conidia and culture filtrates of *T. asperellum* strains controlled rot on banana fruits. *Trichoderma asperellum* NG-T161 has a potential of being used to develop a biopesticide which would be useful in the control of postharvest fruit rot pathogens of *Musa* spp. fruits.

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POLISH SUMMARY

ZWALCZANIE POZBIOROWYCH ZGNILIZN OWOCÓW BANANA KONIDIAMI I FILTRATAMI KULTUR *TRICHODERME ASPERELLUM*

Banany łatwo psują się i są wrażliwe na porażenie mikroorganizmami wywołującymi istotne szkody. Pestycydy używane do zwalczania infekcji mogą być toksyczne dla człowieka i zwierząt. Jest więc zapotrzebowanie na metody zwalczania patogenów przyjazne środowisku. Jednocześnie inokulacja bananów gatunkiem *Trichoderma* i patogenami wywołującymi zgniliznę owoców powodowała ich gnicie, ale gnicie wywołane tylko przez *T. asperellum* NG-T161 lub w połączeniu z patogenem było ograniczone. Traktowanie owoców konidiami lub filtratami kultur *T. asperellum* NG-T161 w ciągu 30 minut przed inokulacją patogenami dawało lepsze wyniki w porównaniu do łącznego zastosowania patogena i antagonisty. Ze zgnitych skrawków tak potraktowanej tkanki owoców wyłożonych na płytki izolowano tylko gatunek *Trichoderma*. Filtraty kultur *T. asperellum* w stężeniu 50% inhibowały wzrost grzybni *F. oxysporum* i *Colletotrichum musae* odpowiednio o 49,7 i 60,3%, ale grzyb *Lasioidiplodia theobromae* nie był inhibowany. Szczepy *T. asperellum* były pasożytnicze w stosunku do patogenów powodujących gnicie bananów. Konidia i filtraty kultur *T. asperellum* NG-T161 zwalczały zgniliznę bananów. Grzyb ten mógłby być wykorzystany do opracowania biofungicydu do zwalczania patogenów wywołujących gnicie bananów.