

Invitro Screening of Trichodermaisolates for Biological Control of A Post-Harvest Rot Fungus

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Abstract: Seven isolates of *Trichoderma* species were evaluated invitro for biological control activity against a post-harvest rot causing strain of *A. niger*. Simultaneous pairing and culture filtrate assays were carried out and readings of growth inhibitions taken at 48 hour intervals for 5-9 days. *T. hamatum* gave the highest growth reduction (57.06%) of *A. niger* in simultaneous pairing, followed by *T. harzianum* (51.47%). *T. longibrachiatum* T2 recorded the least radial growth inhibition of *A. niger* (23.53%). Significant differences in growth inhibition ($P < 0.05$) were observed only after 24 hours post-pairing. Zones of growth inhibition 1-3mm wide were also observed in pairing with *T. hamatum*, *T. pseudokoningii* and *T. polysporum*. Effect of carbon source on antagonistic properties of culture filtrates of *Trichoderma* species on *A. niger* indicated highest and significantly different ($P < 0.05$) radial growth reduction of *A. niger* by casein-based culture filtrates of *Trichoderma* (66.35%), followed by Cmc Cellulose (15.76%) and Prawn waste (15.71%) - based culture filtrates respectively. Results of overall antagonistic activity of *Trichoderma* species on *A. niger* indicated a higher growth inhibition of *A. niger* by *T. harzianum* (42.73%) followed by *T. polysporum* (42.47%). The least overall antagonistic effect on the test organism was by *T. longibrachiatum* T1 (20.45%). Differences in overall inhibitory activity of *A. niger* by *Trichoderma* species were not significantly different ($P < 0.05$). *Trichoderma* isolates used in the study yielded significant growth inhibitions of the test fungus and could be considered in the biological control of post-harvest rot of stored products caused by *A. niger*.

Key words: *Trichoderma*, Culture filtrates, Simultaneous pairing, *A. niger*, Post-harvest rot.

I. Introduction

Microorganisms threaten food security annually, fungi being the most aggressive [1]. To maintain the quality and abundance of food, feed, and fiber by growers globally, affordable, sustainable and environmentally friendly approaches to control of plant pathogenic fungi are sacrosanct [2,3,4].

The term biological control in plant pathology applies to the use of microbial agents to mitigate disease as well as suppress weed populations in order to improve crop yield and preserve stored products [5]. Currently, the role of biocontrol agents is a well-established fact and has become increasingly crucial. In several cases, they complement or even replace their chemical counterparts, where antagonistic fungi play an important role [6].

In the context of biological control of plant pathogens by fungal agents, *Trichoderma* species have become the cynosure of many researchers seeking to contribute to the biocontrol endeavor through the use of fungi [6]. The potential of *Trichoderma* species as biological control agents of plant diseases was first recognized in the early 1930's and since then, control of many diseases and pathogens has been achieved [7, 8,9,10,11].

Disease control by *Trichoderma* species is achieved through the activation of a myriad of mechanisms, direct and indirect [1]. Direct mechanisms of pathogen and disease control by *Trichoderma* species include mycoparasitism through synthesis of lytic enzymes [1, 12,13]. Mycoparasitism has been proposed as the central mechanism responsible for the antagonistic activity of *Trichoderma* species [14]. Indirect mechanisms include competition and rhizosphere competence, Induction of defence responses in plants, biofertilization and antibiosis [9,15, 16].

The research reported in this paper was aimed at screening a group of *Trichoderma* isolates for biological control potentials against a post-harvest rot strain of *Aspergillus niger*, a common pathogen of stored agricultural products in the study area.

II. Materials and Methods

2.1. Source and Identification of Isolates

2.1.1.A. niger

The post-harvest rot strain of *A. niger* used in the study was generously donated by a fellow researcher, Mr. Tivkaa Amade of the Department of Microbiology, University of Uyo, Nigeria.

2.1.2. *Trichoderma* Species

Trichoderma species were isolated from farm and refuse dump soils in Ibadan, Nigeria, using the serial dilution plate technique on Potato Dextrose Agar (PDA), at concentration 10^{-6} . *Trichoderma* species were identified with the aid of identification keys of Rifai [17] and the compendium of soil fungi [18], using morphological and microscopic examination of purified isolates.

2.2. Growth Conditions For Production of Antifungal Metabolites By *Trichoderma* species

2.2.1. Casein as Carbon Source

The growth medium was formulated as reported by De Marco and Felix [14] with slight modifications as follows: Bactopeptone (0.1%), Urea (0.03%), KH_2PO_4 (0.2%), $(\text{NH}_4)_2\text{SO}_4$ (0.14%), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.03%), $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ (0.03%), Casein (0.5%), Glucose (0.02%), 1ml of 0.01% trace elements solution (Fe^{2+} , Mn^{2+} , Zn^{2+} , and CO_2), pH 5.5 (0.2M Sodium acetate buffer).

2.2.2. Prawn waste as carbon source

A modified medium of Kukuc and Kivanc [2] was used as follows (g/l of distilled water): $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; KH_2PO_4 , 0.9; KCl, 0.2; NH_4NO_3 , 1.0; Fe^{2+} , 0.002; Zn^{2+} , 0.002; 10g of finely sieved prawn waste powder (0.4mm mesh size), pH 5.5 (0.2M Sodium acetate buffer).

2.2.3. Cmc cellulose as carbon source

The medium of Mendels and Weber [19] was used as follows (g/l of distilled water): Cellulose powder, 10; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; KH_2PO_4 , 2.0; $(\text{NH}_4)_2\text{SO}_4$, 1.4; Urea, 0.3; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.4; peptone, 0.75; yeast extract, 0.25; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.005; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.0016; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0014; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.02, pH 5.5 (0.1M Sodium citrate buffer).

2.3. Culture of Antagonists and Collection of Culture Filtrates

Three 7mm mycelia plugs from actively growing regions of 3-5 days old cultures of antagonist were aseptically obtained and introduced into 15ml volume of each sterilized growth media in McCartney bottles. Cultures were incubated at 30°C for a total duration of 5 days after which mycelia and conidia were removed via filtration using a double membrane Watman no.1 filter paper. Centrifugation of filtrates was carried out at 8000rpm for 10mins at 10°C in a high speed refrigerated centrifuge. Carefully decanted supernatants were probe of antagonistic activity.

2.4. Determination of Antagonistic Activity of Culture Filtrates of *Trichoderma* Species

The method reported by El-Katatny *et al.* [20] was employed. Sterilized molten PDA ($\frac{1}{4}$ strength) was aseptically mixed with 10% concentrations of various culture filtrates of *Trichoderma* species in 8.5mm diameter petri dishes and allowed to solidify. Plates were inoculated separately at the center with 5mm diameter mycelia disc of *A. niger*, obtained from actively growing regions of 2-7 days old cultures and incubated at 30°C for 5 days. Radial growth of the pathogen was taken as the average of colony diameter measured at right angles, and percentage pathogen growth inhibition calculated with respect to radial growth of pathogen on control plates (without culture filtrates) as follows:

$$\text{PRG} = \frac{\text{PC} - \text{P}}{\text{PC}} \times 100 \quad (1)$$

Where, PRG = Percentage Radial Growth Inhibition of *A. niger*; Pc = Radial growth of *A. niger* in control plate; P = Radial growth of *A. niger* in the presence of culture filtrates of *Trichoderma* species.

2.5. Dual Culture Tests

5mm diameter mycelia plugs of *Trichoderma* species and *A. niger* obtained from actively growing regions of 2-7 days old cultures on PDA, were simultaneously plated 3mm apart on $\frac{1}{4}$ strength PDA in 8.5mm diameter petri dishes and incubated at 30°C for 9 days. Plates were observed every 48 hours for growth inhibitory activity. 5mm diameter mycelia plug of *A. niger* was cultured in a separate 8.5mm diameter petri dish in the absence of *Trichoderma* species as control. The radial diameter of fungal colonies was measured at right angles and percentage radial growth inhibition determined as earlier described in equation (1) above.

2.6. Experimental Design

The experiment was set up in Completely Randomized Design (CRD) and treatments administered in two replicates.

2.7. Data Analysis

All data collected were subjected to Analysis of Variance (ANOVA) and the means separated using Duncan's Multiple Range Test (DMRT) at P<0.05.

III. Results

Table 1. Radial growth inhibition of *A. niger* by various culture filtrates of *Trichoderma* species after 5 days of post-inoculation.

<i>Trichoderma</i> species	% Radial Growth Reduction		
	Filtrate A	Filtrate B	Filtrate C
<i>Trichoderma</i> sp. T1	4.55 ^c	5.37 ^c	87.60 ^a
<i>T. hamatum</i>	-2.89 ^c	7.44 ^c	85.54 ^{ab}
<i>T. longibrachiatum</i> T1	30.58 ^b	30.17 ^b	58.68 ^{ab}
<i>T. pseudokoningii</i>	-8.27 ^c	4.13 ^c	73.97 ^a
<i>T. harzianum</i>	20.25 ^b	13.64 ^c	85.54 ^{ab}
<i>T. longigrachiatum</i> T2	2.89 ^c	3.72 ^c	51.65 ^{ab}
<i>T. polysporum</i>	62.81 ^a	45.87 ^a	21.49 ^b
Average total	15.71 ¹	15.76 ¹	66.35 ²

Means followed by same alphabetic superscripts within a column are not significantly different (P<0.05)

Means followed by different alphabetic superscripts within a column are significantly different (P<0.05)

Means followed by same numeric superscripts within a row are not significantly different (P<0.05).

Means followed by different numeric superscripts within a row are significantly different (P<0.05).

Filtrate A = Filtrate derived from use of prawn waste as carbon source

Filtrate B = Filtrate derived from use of CMC cellulose as carbon source

Filtrate C = Filtrate derived from use of casein as carbon source

Results of antagonistic activity of various culture filtrates of *Trichoderma* species on growth of *A. niger* (TABLE 1) indicates highest and significantly different (P<0.05) radial growth reduction of *A. niger* by casein based culture filtrates of *Trichoderma* sp. (66.35%), followed by Cmc Cellulose (15.76%) and Prawn waste (15.71%) based culture filtrates respectively. Radial growth of *A. niger* was inhibited the most by culture filtrates of *Trichoderma* sp. T1 (87.60%), followed by both *T. hamatum* (85.54%) and *T. harzianum* (85.54%), all with casein as carbon source. Growth inhibitions of *A. niger* by culture filtrates of *Trichoderma* sp. T1 differed significantly (P<0.05) from other *Trichoderma* species.

Table 2. Radial growth inhibition of *A. niger* by *Trichoderma* species in dual cultures

<i>Trichoderma</i> species	% Radial Growth Reduction				
	Day 1	Day 3	Day 5	Day 7	Day 9
<i>Trichoderma</i> sp. T1	3.08 ^g	7.23 ^a	21.97 ^a	31.35 ^a	35.29 ^a
<i>T. hamatum</i>	16.92 ^h	28.31 ^a	45.83 ^a	53.29 ^a	57.06 ^a
<i>T. longibrachiatum</i> T1	-3.08 ^c	15.06 ^a	28.41 ^a	40.44 ^a	44.71 ^a
<i>T. pseudokoningii</i>	-6.15 ^b	7.83 ^a	35.61 ^a	46.40 ^a	48.24 ^a
<i>T. harzianum</i>	-1.54 ^d	12.05 ^a	37.88 ^a	47.96 ^a	51.47 ^a
<i>T. longigrachiatum</i> T2	1.54 ^f	16.87 ^a	25.0 ^a	20.69 ^a	23.53 ^a
<i>T. polysporum</i>	0.00 ^e	18.68 ^a	31.06 ^a	35.74 ^a	39.71 ^a

Means followed by same superscripts within a column are not significantly different (P<0.05)

Means followed by different superscripts within a column are significantly different (P<0.05)

Simultaneous pairing of *Trichoderma* species with *A. niger* (TABLE 2) yielded a steady increase in percentage radial growth reduction of *A. niger* from day 1 to day 9. *T. hamatum* gave the highest growth reduction (57.06%) of *A. niger* followed by *T. harzianum* (51.47%). *T. longibrachiatum* T2 recorded the least radial growth inhibition of *A. niger* (23.53%). Significant differences in growth inhibition (P<0.05) were observed only after 24 hours post-pairing. Zones of growth inhibition 1-3mm wide were also observed in pairing with *T. hamatum*, *T. pseudokoningii* and *T. polysporum* (Plate 1).

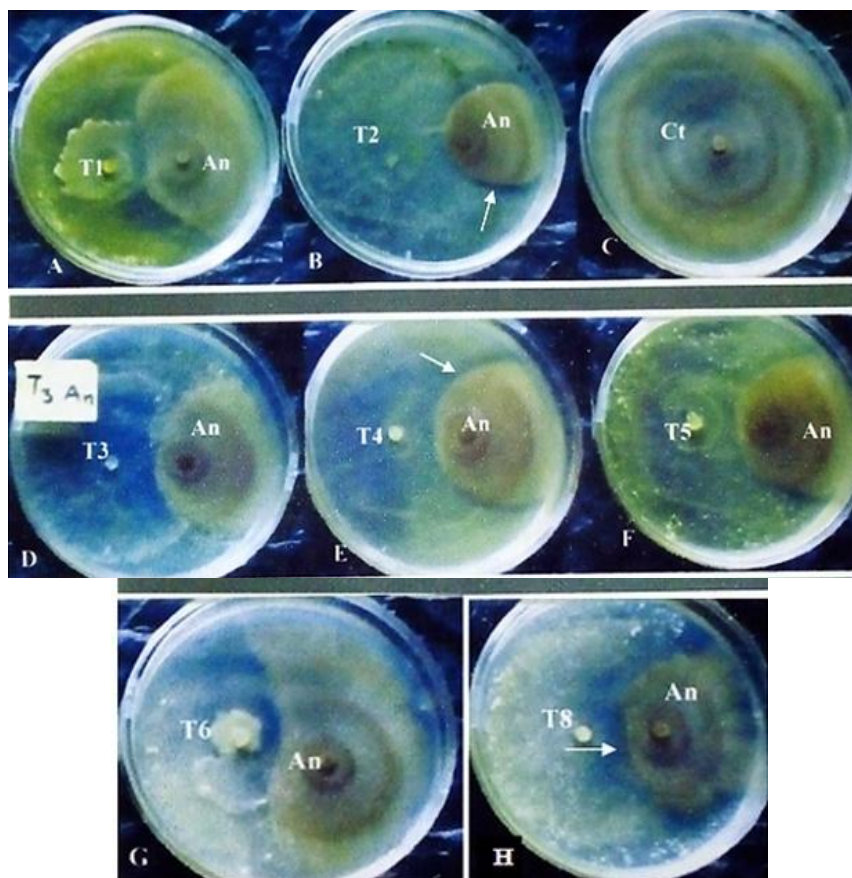


Plate 1. Simultaneous pairing of *Trichoderma* species with *Aspergillus niger* indicating zones of growth inhibition (See arrows) after 9-11 days of post-inoculation.

T1= *Trichoderma* sp. T1
 T2 = *T. hamatum*
 T3 = *T. longibrachiatum* T1
 T4 = *T. pseudokoningii*
 T5 = *T. harzianum*
 T6 = *T. longibrachiatum* T2
 T8 = *T. polysporum*
 An = *A. niger*
 Ct = Control

Table 3. Overall growth reduction of *A. niger* by different antagonistic mechanisms of *Trichoderma* species.

Mechanism	Growth inhibition (%)
Simultaneous pairing (Competition)	42.84 ^a
Culture filtrates (Antibiosis)	66.35 ^b

Means followed by same superscripts within a column are not significantly different (P<0.05)
 Means followed by different superscripts within a column are significantly different (P<0.05)

A comparison of the different growth inhibitory mechanisms assayed for in this study (TABLE 3) indicates a higher and significantly different radial growth inhibition of *A. niger* by culture filtrates of *Trichoderma* species (66.35%) compared to simultaneous pairing (42.84%).

Table 4. Overall antagonistic activity of *Trichoderma* species on *A. niger*

<i>Trichoderma</i> species	Antagonistic activity (%)
<i>Trichoderma</i> sp.T1	33.20 ^a
<i>T. hamatum</i>	36.79 ^a
<i>T. longibrachiatum</i> T1	41.04 ^a
<i>T. pseudokoningii</i>	29.52 ^a
<i>T. harzianum</i>	42.73 ^a
<i>T. longibrachiatum</i> T2	20.45 ^a
<i>T. polysporum</i>	42.47 ^a

Means followed by same superscripts within a column are not significantly different (P<0.05)

An assessment of overall antagonistic activity of *Trichoderma* species on *A. niger* (TABLE 4) indicates a higher growth inhibition of *A. niger* by *T. harzianum* (42.73%) followed by *T. polysporum* (42.47%). The least overall antagonistic effect on the test organism was by *T. longibrachiatum* T1 (20.45%). Differences in overall inhibitory activity of *A. niger* by *Trichoderma* species were not significantly different (P<0.05).

IV. Discussion

Trichoderma species used in the study exhibited various levels of growth inhibition of the rot fungus *A. niger*. The ability of *Trichoderma* species to inhibit growth of pathogenic fungi has been accounted for by several authors. Their mechanisms of antagonism include mycoparasitism through the synthesis of lytic enzymes that degrade fungal cell walls, competition for space and nutrients, antibiosis, induction of host defence responses and biofertilisation [9,15,16,11].

Dual cultures of the pathogen with *T. hamatum* yielded a substantially higher growth inhibition of the pathogen in comparison with pairing with other *Trichoderma* species. The ability of *T. hamatum* to effectively outgrow and compete favourably with other fungal pathogens for space and nutrients had been earlier reported by Fakhrunnisa *et al.*[21].

Zones of growth inhibition were also observed in simultaneous pairing of *A. niger* with *T. hamatum*, *T. pseudokoningii* and *T. polysporum*. Zones of growth inhibition are indicative of the ability of *Trichoderma* species to secrete toxic substances in advance of contact with potential competitors in a given substrate. Similar observations have been reported in works of several researchers [22,21,23].

Effect of culture filtrates on growth of *A. niger* was significantly influenced by the carbon source used. Similarly, Siameto *et al.*[24] also reported that culture filtrates of *Trichoderma* species obtained from Czapek's media produced higher reduction of mycelial dry weight of soil borne pathogens compared to culture filtrates from Potato Dextrose Broth (PDB). Naher *et al.* [25] also reported variations between antagonistic activity of *Trichoderma* species grown on Potato Sucrose Agar (PSA) and those grown on Potato Dextrose Agar (PDA).

Growth of *A. niger* was inhibited more by culture filtrates than by simultaneous pairing. In a similar work, Padmaja *et al.*[23] also observed that at 50% concentration (v/v), the culture filtrates of a native *Trichoderma* species inhibited growth of *S. rolfii* by 100% compared to 80% inhibition in dual culture. Culture filtrates contain toxic secondary metabolites synthesized by *Trichoderma* species to impede growth of other microbes as a mechanism for effective competition for space and nutrients [1].

T. harzianum was comparatively, the most effective antagonist against *A. niger* in the reported study. The biological control potential of various *T. harzianum* strains have been reported variously by a number of researchers. In a similar work by Siameto *et al.*[24], it was reported that both mycelia growth and culture filtrates of *Trichoderma harzianum* significantly reduced growth of some soil borne fungal pathogens in Kenya.

V. Conclusion

Trichoderma species used in this study demonstrated potent biocontrol qualities and could be further exploited in the sustainable management of post-harvest storage rot by *A. niger* strains.

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