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*Full Length Research Paper*

# Growth inhibition (*in vitro*) of *Colletotrichum gloeosporioides* isolated from cassava (*Manihot esculenta*) using *Trichoderma longibrachiatum*

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**The growth inhibition of *Colletotrichum gloeosporioides* isolated from cassava was studied *in-vitro* using *Trichoderma longibrachiatum*. Both fungi were cultured together on the same Petri plate using three different pairing methods. Inoculation of each fungus on separate Petri plates served as controls. For each pairing method, experiments were conducted in five replicates. Radial growth (cm) of both *C. gloeosporioides* and *T. longibrachiatum* in all Petri plates were measured daily for 7 days. In all the three pairing methods, *T. longibrachiatum* significantly inhibited the growth of *C. gloeosporioides* (P > 0.0001). Growth inhibition of *C. gloeosporioides* by *T. longibrachiatum* was better in 'inoculating antagonist before pathogen' than in the other two pairing methods (P = 0.05). *C. gloeosporioides* had significant addition of radial mycelia only between days 1 and 2, as well as days 2 and 3 after pairing (DAP) before contact was made with *T. longibrachiatum.* Addition of mycelia mass of *C. gloeosporioides* slowed down significantly by the day upon contact with *T. longibrachiatum* (P = 0.05, $R^2$ = 0.86). F value for day after pairing (DAP) was also highly significant (P > 0.0001). *T. longibrachiatum* could thus be said to possess probable antagonistic tendency against *C. gloeosporioides.***

**Key words:** *Colletotrichum gloeosporioides*, *Trichoderma longibrachiatum*, *Manihot esculenta,* day after pairing, growth inhibition.

## INTRODUCTION

Cassava, *Manihot esculenta* (Crantz), of the root and tuber crops grown in Africa remains a major source of carbohydrate. The starchy root crop, which is among the most important tropical food crops (Cock, 1985; Owolade et al., 2008), has over 600 million people depending on it in Africa, Asia and Latin America for food security and income generation (FAO, 2002). Fungal diseases amongst others pose serious constraints to the crop all over the world. Some of such diseases are so devastating that they allow little or no yield of the root crop if not controlled. *Colletotrichum gloesosporioides* f.sp. *manihotis* is known as a major pathogen of the crop causing cassava anthracnose, which is regarded as the most important fungal disease of cassava in the field (Hahn et al., 1989; Fokunang, 1997). In Congo and Zaire, 80 - 90% of local cultivars of cassava were recorded as being severely infected by the fungus (Muyolo, 1984). *C. gloesosporioides* has also been well documented to be the major cause of yam (*Dioscorea sp*.) anthracnose (Amusa, 2000). Infection by the fungus is known to cause epidemic disease that is characterized by particular symptoms such as cankers on stems, branches and

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fruits, leaf spots and shoot die back (Muimba, 1982; Theberge, 1985; IITA, 1990; Ogbebor et al., 2007). Symptomless cassava materials can also contain *C. gloeosporioides* f.sp. *manihotis* that can only manifest itself under favourable environmental conditions (Fokunang et al., 2000). The fungus is however, relatively inactive in dry weather, low humidity and temperature extremes (Dickman and Alvarez, 1982).

The use of antagonistic microorganisms in controlling plant diseases continues receiving increasing attention (Adebanjo and Bankole, 2004). Successful use of the genus *Trichoderma* in the biological control of several plant pathogens has also been well documented (Paavanen-Huhtala et al., 2000). *Trichoderma* species have provided varied level of biological control of a number of important pathogens, both *in vitro* and *in vivo* (Smith et al., 1990; Sobowale et al., 2005, 2007). This work preliminarily examines the ability of *Trichoderma longibrachiatum* in inhibiting growth of the pathogen *C. gloeosporioides in vitro*.

## MATERIALS AND METHODS

### Collection of *C. gloeosporioides* and *T. longibrachiatum*

Culture of *C. gloeosporioides* was collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Culture of *T. longibrachiatum* was obtained from the Department of Plant Science and Applied Zoology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

### Culturing *T. longibrachiatum* with *C. gloeosporioides*

In culturing *T. longibrachiatum* with the pathogen (*C. gloeosporioides*), three pairing methods were used, with each fungus inoculated at opposite ends of 9 cm Petri plate containing 15 ml acidified potato dextrose agar (Sobowale et al., 2005). In the first pairing method, *T. longibrachiatum* was inoculated 24 h before *C. gloeosporioides* (AGxb4P). In the second, *C. gloeosporioides* was inoculated 24 h before *T. longibrachiatum* (Pb4AGx), while both fungi were simultaneously inoculated in the third pairing method (AGxP). Inoculation of *C. gloeosporioides* only and *T. longibrachiatum* only in separate Petri plates served as controls. For each pairing method, experiments were conducted in five replications. All Petri plates were incubated at 28°C for 7 days.

### Data collection and analysis

Daily measurements of radial growth (cm) of both *C. gloeosporioides* and *T. longibrachiatum* in all Petri plates were taken for 7 days. The resulting values were analysed using the Generalized Linear Model (GLM Procedure) of SAS (SAS, 1989).

## RESULTS

### Growth inhibition of *C. gloeosporioides* by *T. longibrachiatum*

When *T. longibrachiatum* was inoculated before *C. gloeosporioides*, the *Trichoderma* species grew fast colonizing the subsurface of the acidified potato dextrose agar (APDA) Petri plate by a light greenish metabolite ahead of its surface mycelia growth, thereby restricting growth of *C. gloeosporioides* around inoculation point within 4 days after pairing. By the 6th day after pairing, *T. longibrachiatum* had begun growing on top of hyphae of *C. gloeosporioides*. By the 7th day, the Petri plates almost appeared as pure cultures of *T. longibrachiatum*, thus making re- isolation of *C. gloeosporioides* difficult (Figure 1). There was no clear zone of inhibition.

On inoculating *C. longibrachiatum* before *T. longibrachiatum*, the *Trichoderma* grew fast, covering much space within three days, slowing down growth of *C. gloeosporioides* by the 4th day after pairing. By the 6th day after pairing, *T. longibrachiatum* had started sporulatimg on top of mycelia of *C. longibrachiatum* (Figure 2). There was no clear zone of inhibition. When both fungi were inoculated simultaneously, *T. longibrachiatum* still grew faster than *C. gloeosporioides*, covering much space on Petri plate and considerably reducing growth rate of the pathogen (*C. gloeosporioides*) by the 3rd day after pairing. By the 6th day, in similar manner as in other two pairing methods, *T. longibrachiatum* had started sporulating heavily on mycelia of *C. gloeosporioides* , making re- isolation of the pathogen difficult (Figure 3). There was also no clear zone of inhibition. In all pairing methods, contact was made between both fungi by the 3rd day after pairing.

### Analysis of growth performance of *T. longibrachiatum* against *C. gloeosporioides*

In all pairing methods (Table 1), radial growth of *C. gloeosporioides* was significantly different from control ($P = 0.05$). *T. longibrachiatum* also had better growth inhibition of *C. gloeosporioides* ($P = 0.05$) in 'inoculating antagonist before pathogen' than in the other two pairing methods. Same was the case in 'simultaneous inoculation of both fungi' where growth inhibition of *C. gloeosporioides* was significantly better than in 'inoculating pathogen before antagonist' (Table 1). The pathogen showed significant addition of radial mycelia mass between days 1 and 2, as well as days 2 and 3. It however, slowed down in rate of mycelia addition in subsequent days (Table 2). F values (Table 3) were highly significant ($P > 0.0001$) for the model, pairing method, and days after pairing (DAP).

## DISCUSSION

The ability of *T. longibrachiatum* to grow and cover more space living little space for growth of *C. gloeosporioides* is due to the high sporulation capacity that is generally characteristic of the genus *Trichoderma*

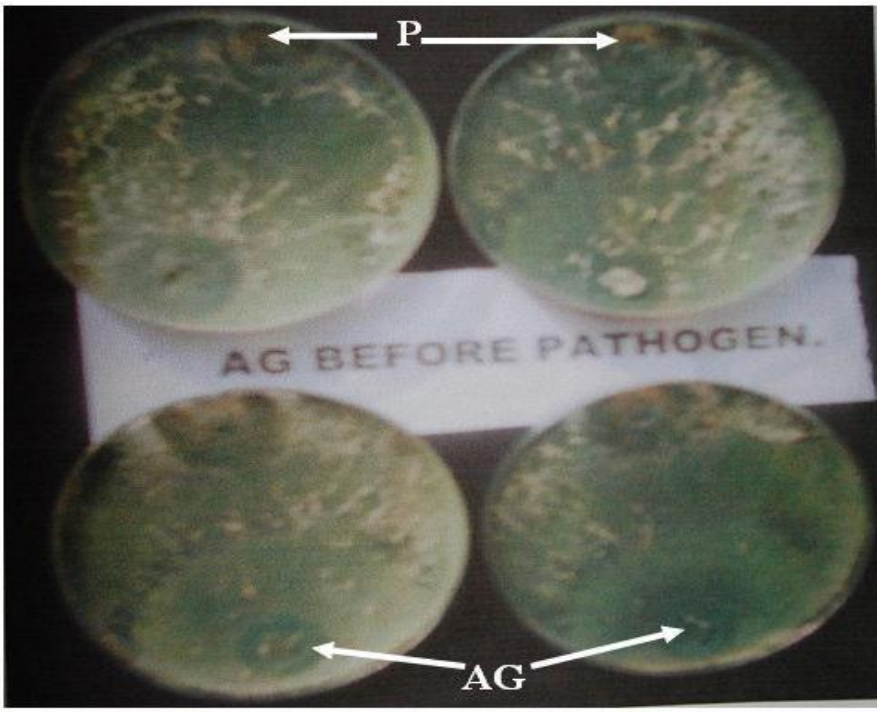


**Figure 1.** Inoculation of *T. longibrachiatum* (AG) 24 h before *C. gloesporioides* (P). The pathogen (P) is restricted to point of inoculation (topside) and all plates later appeared as pure culture of *T. longibrachiatum*.

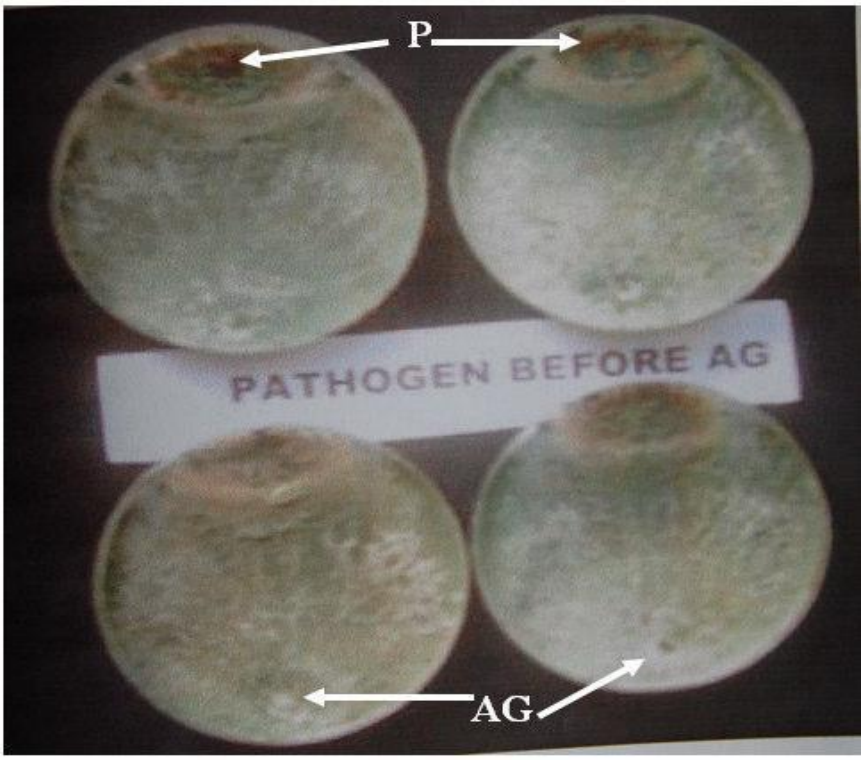


**Figure 2.** Inoculation of *C. gloesporioides* (topside) 24 h before *T. longibrachiatum*. Mycelia of pathogen is seen (topside) completely overgrown by that of *T. longibrachiatum.*

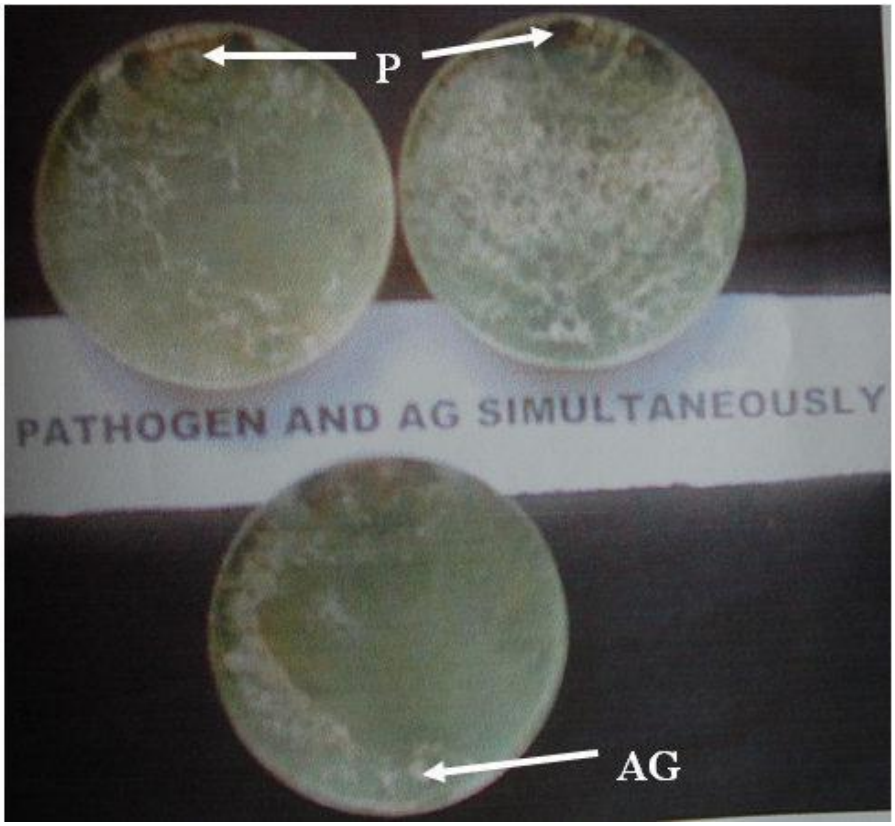


**Figure 3.** Simultaneous inoculation of *T. longibrachiatum* (AG) and *C. gloeosporioides (*P). The pathogen (topside) is seen completely overgrown by *T. longibrachiatum.*

**Table 1.** Means of radial growth of *C. gloeosporioides* in pairing with *T. longibrachiatum*.

| Pairing method | Means of pathogen's radial growth |
|---|---|
| Pathogen inoculated alone (Control) | 3.99a |
| Pathogen inoculated before antagonist | 2.29b |
| Antagonist and pathogen inoculated simultaneously | 2.08c |
| Antagonist inoculated before pathogen | 1.68d |
| $LSD_{0.05}$ | 1.03 |

Means with different letters are significantly different from each other.

**Table 2.** Means comparison of radial growth of *C. gloeosporioides* in presence of *T. longibrachiatum* daily for 7 days.

| Day after pairing (DAP) | Pathogen alone (control) | Means of radial growth of pathogen in presence antagonist |
|---|---|---|
| 1 | 0.92a | 0.93a |
| 2 | 1.38b | 1.36b |
| 3 | 1.91c | 1.79c |
| 4 | 2.34d | 2.11cd |
| 5 | 2.96e | 2.39de |
| 6 | 3.42f | 2.62ef |
| 7 | 3.87g | 2.86f |
| $LSD_{0.05}$ | 0.33 | 0.33 |
| $R^2$ | | 0.86 |

Means with different letters are significantly different each other.

**Table 3.** Anova table for radial growth of *C. gloeosporioides* in presence of *T. longibrachiatum*.

| Source | DF | MS | F-value | P>F |
|---|---|---|---|---|
| Model | 14 | 25.80 | 71.98 | 0.0001* |
| Pairing method | 4 | 71.29 | 198.87 | 0.0001* |
| DAP | 6 | 12.12 | 33.81 | 0.0001* |
| Rep | 4 | 0.84 | 2.35 | 0.056 |
| Error | 160 | 0.36 | | |
| Total | 174 | | | |

DAP= Day after pairing, ** = highly significant.

(Paavanen-Huhtala et al., 2000). Its ability to first colonize the subsurface of the agar, well before growing on the agar surface also aided its competitive strength for space and nutrients against *C. gloeosporioides*. The light greenish excretion released into the agar by *T. longibrachiatum* could be a toxic metabolite (to *C. gloeosporioides*) that went ahead of the mycelia growth of the *Trichoderma*, restricting growth of *C. gloeosporioides* around inoculation point. This suggests possibility of antibiosis as one mode of antagonism here, although, more work needs to be done before credible conclusion can be drawn on this. Production of fungitoxic metabolite is known to be a common phenomenon with the genus *Trichoderma* (Thrane et al., 2000). Complete obliteration of *C. gloeosporioides* by the fast growing mycelia of *T. longibrachiatum* on the 7th day after pairing, irrespective of pairing method could be suggesting mycoparasitism as another probable mode of inhibition. However, this also, can only be ascertained after further experiments. The significant reduction in radial mycelia growth of *C. gloeosporioides* in presence of *T. longibrachiatum* (compared to control), in all pairing methods (Table 1) showed the potential of the latter in suppressing growth of *C. gloeosporioides*. The significant difference in growth performance of *T. longibrachiatum* against that of *C. gloeosporioides* among pairing methods (Table 1) showed that pairing method impacts significantly on performance of the *Trichoderma* against *C. gloeosporioides*. The significant effect of pairing method is also shown by the highly significant F value (P > 0.0001) for pairing method (Table 3). This underscored the significant impact of pairing method on growth inhibition of *C. gloeosporioides* by *T. longibrachiatum*. The significant reduction in daily addition of mycelia of *C. gloeosporioides* from the 4th DAP (Table 2) showed the probable inhibitory effect of *T. longibrachiatum* on *C. gloeosporioides* upon contact of both fungi. This inhibitory effect is further made evident by the significant addition of radial mycelia of *C. gloeosporioides* on a daily basis in the absence of *T. longibrachiatum* (control, Figure 4), as well as between days 1 and 3 after pairing, before contact with *T. longibrachiatum* (Table 2).

High significant F value for DAP (P > 0.0001) thus suggests the importance of pairing duration to effective

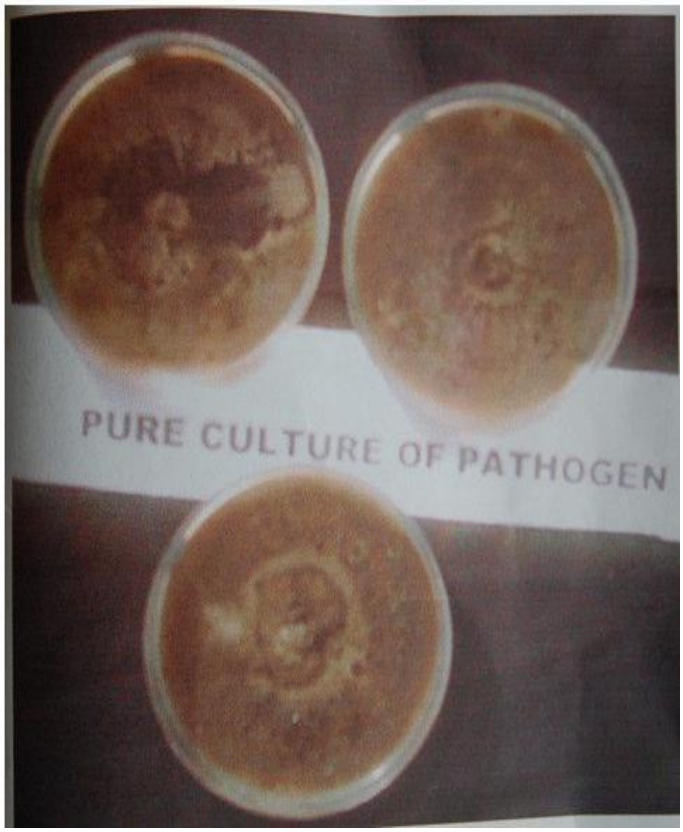


**Figure 4.** Rapid spread of *C. gloeosporioides* in the absence of *T. longibrachiatum*.

growth inhibition of *C. gloeosporioides* by *T. longibrachiatum* (Table 3). It strongly suggests that the longer the duration of pairing (DAP) *C. gloeosporioides* with *T. longibrachiatum*, the better the antagonistic potential of the *Trichoderma* against the pathogen. It explains the down trend in rate of mycelia addition of *C. gloeosporioides* (upon contact with *T. longibrachiatum*) which reduces with increase in DAP (Table 2).

Although, further experiments must be conducted before credible statements could be made on antagonistic capability of *T. longibrachiatum* against *C. gloeosporioides*, the *T. longibrachiatum* could still be said to possess promising traits of antagonism against *C. gloeosporioides* just like it showed against *Botryodiplodia theobromae* (Sobowale et al., 2008).

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