

SUPPRESSION OF GROWTH OF *FUSARIUM VERTICILLIOIDES* NIREN. USING STRAINS OF *TRICHODERMA HARZIANUM* FROM MAIZE (*ZEA MAYS*) PLANT PARTS AND ITS RHIZOSPHERE

Ayodele Adegboyega Sobowale^{1*}, Adegboyega Christopher Odebode²
Kitty Frances Cardwell³, Ranajit Bandyopadhyay⁴

¹Department of Plant Science and Applied Zoology, Olabisi Onabanjo University, P.M.B. 2002, Ago-Iwoye, Ogun State, Nigeria

²Department of Botany and Microbiology, University of Ibadan, Ibadan, Oyo-State, Nigeria

3800 9th St SW rm 3436, Washington DC 20024, USA

kittycardwell@hotmail.com, kcardwell@csrees.usda.gov

⁴Pathology Unit, International Institute of Tropical Agriculture, c/o L.W. Lambourn (UK) Ltd, Carolyn House, 26 Dingwall Road, Croydon CR9 3EE, UK, R.Bandyopadhyay@cgiar.org

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Abstract: Three strains of *Trichoderma harzianum* (strain 1: IMI 380934; strain 2: IMI 380935; strain 3: IMI 380938) were compared for their ability to suppress radial growth of *Fusarium verticillioides* *in vitro*. Each *Trichoderma* strain was paired with the pathogen *F. verticillioides* on 9 cm Petri plates of acidified potato dextrose agar using three pairing methods. Varying growth suppression of pathogen by *Trichoderma* strains was rated and ratings were analysed using GLM Procedure of SAS. Growth inhibition of *F. verticillioides* by each of the *T. harzianum* strains was significantly different from control irrespective of pairing method ($p = 0.01$, $R^2 = 0.96$). Higher inhibition of *F. verticillioides* was obtained by inoculating antagonist before pathogen even at $p = 0.01$. Mode of suppression includes mycoparasitism and competition for space and nutrients. Growth inhibition of pathogen differed significantly among ($p > 0.0001$) and within ($p > 0.026$) pairing methods. *T. harzianum* strain 1 had better suppression of pathogen than the other two strains when it was inoculated before the pathogen while *T. harzianum* strain 3 was better when pathogen and antagonist were inoculated simultaneously ($p = 0.05$). Different strains of *T. harzianum* could thus be employed as promising antagonists of *F. verticillioides*.

Key words: antagonist, *Fusarium verticillioides*, pathogen, *Trichoderma harzianum*, strain

INTRODUCTION

Among major cereal crops in the world production, maize (*Zea mays*) was documented to rank third following closely behind wheat and rice (Bunting *et al.* 1978). It could easily pass as the world's most widely distributed crop with a relatively lower price compared to other cereals. It could thus be said to enjoy wider range of uses than any other cereals (Bunting *et al.* 1978). *Fusarium verticillioides* (formerly *F. moniliforme*) is one important pathogen known to be a constant companion of maize with the ability to cause disease at all stages of maize plant development (Kedera *et al.* 1992). Apart from maize, it actually infects a wide range of other crops like sorghum, wheat, barley, etc. worldwide (Visconti and Doko 1994; Soonthornpocet *et al.* 2000). It is not only the most common pathogen of maize, but also the most common fungus colonizing symptomless maize plants (Munkvold and Desjardins 1997). It has been implicated in the stem rot of maize (*Zea mays*). It is known to be mycotoxigenic and carcinogenic, posing great threats to human and animal health (Marasas 1988; Julian *et al.* 1995).

Trichoderma is a unique genus that is made up of fungi most commonly used as biocontrol fungi against many pathogens *in vitro* and *in vivo* (Paavanen-Huhtala *et al.* 2000). Mycoparasitism, competition, and antibiosis, amongst others, are different mechanisms by which members of the genus bring about their biocontrol activity (Campbell 1988; Wells 1988; Sharma and Sankaran 1988). Howell (2003) hinged their successful biocontrol records on their ability to parasitize other fungi. Amongst several other reports, *T. viride*, isolated from roots of maize plants was reported to suppress radial colony extension of *F. verticillioides* *in vitro* (Yates *et al.* 2000). *T. harzianum* amongst others are also reported to be effective in controlling pigeon pea wilt of *Fusarium oxysporum* f. sp. *udum* (Somasekhara *et al.* 1996). The *in vitro* experiment was a preliminary study to examine effectiveness of *T. harzianum* as an antagonist of the maize (*Z. mays*) stem rot pathogen *F. verticillioides* as well as the effect of pairing method on its antagonistic potential.

*Corresponding address:
delesobowale@yahoo.com

MATERIALS AND METHODS

Isolating and identifying *T. harzianum* and *F. verticillioides*

Naturally infected maize (*Z. mays*) stems, brought into the laboratory from the field were split open longitudinally using sharp sterile knife. Fragments from the rotting parts were prepared and surface sterilized in 1% sodium hypochlorite (for 5 minutes) and later rinsed in five separate beakers with sterile distilled water. Sterile forcep was then used to pick up and place the rotted fragments onto sterile filter papers which were wrapped for 5 minutes. Later the dried fragments were plated in Petri plates with acidified potato dextrose agar (APDA). The plates were incubated at 28–30°C for 10 days. Resulting mixed cultures of fungal colonies were subcultured appropriately to obtain pure cultures. To identify *F. verticillioides*, which forms long chains of conidia on potassium chloride medium (Nelson *et al.* 1983), each *Fusarium* isolate was cultured in Petri plates containing potassium chloride (KCl) medium.

The *Trichoderma* species were isolated from different parts of maize plant and its rhizosphere using stalk sectioning and soil dilution plate method (Tuite 1969). They were sent to International Mycological Institute (IMI) for identification after pure cultures of each fungus was stored on silica gel using the method of Smith and Onions (1983).

Pairing *T. harzianum* strains with *F. verticillioides*

Individual strains of *T. harzianum* (AG) were tried as potential antagonists of pathogen (P) by pairing both fungi *in vitro*. The two fungi were inoculated at opposite ends of 9 cm Petri plate with APDA using three pairing methods. These were: inoculation of antagonist two days before pathogen, AGb4P; inoculation of pathogen two days before antagonist (Pb4AG); and simultaneous inoculation of pathogen and antagonist (AGP) (Sobowale *et al.* 2005). Three replicates were done for each pairing method. All plates were incubated for 30 days at 28–30°C.

Collecting data and statistical analysis

The radial growth of both pathogen and antagonist were measured daily until growth stopped. Varying extent of growth inhibition of *F. verticillioides* by *T. harzianum* strains in Petri plates was estimated using the rating scales of 0–10 viz., 0: if antagonist grows and covers the whole 9 cm plate; 1: if pathogen covers between 0 to 0.90 cm of plate; 2: if pathogen covers between 0.91 to 1.80 cm of plate; 3: if pathogen covers between 1.81 to 2.70 cm of plate; 4: if pathogen covers between 2.71 to 3.60 cm of plate; 5: if pathogen covers between 3.61 to 4.50 cm of plate; 6: if pathogen covers between 4.51 to 5.40 cm of plate; 7: if pathogen covers between 5.41 to 6.30 cm of plate; 8: if pathogen covers between 6.31 to 7.20 cm of plate; 9: if pathogen covers between 7.21 to 8.10 cm of plate; and 10: if pathogen grows between 8.1 cm and the whole 9 cm diameter plate. Resulting values of ratings were analyzed using the General Linear Model option of SAS. This was done to compare relative antagonistic potential of the *T. harzianum* strains against *F. verticillioides*

in vitro and to determine pairing method that best aided effective radial growth inhibition of *F. verticillioides*.

RESULTS

T. harzianum strain 1 (IMI 380934) paired with *F. verticillioides*

After inoculating *T. harzianum* strain 1 two days before *F. verticillioides*, contact was made between them within two days of pairing. By the 4th day of pairing, *T. harzianum* strain 1 grew fast in all Petri plates leaving very little space for growth of pathogen. The pathogen barely had a chance to grow to an average of 1.5 cm diameter. On the 6th day after inoculation, all Petri plates appeared as pure cultures of antagonist (Fig. 1a). There was no zone of inhibition.

After inoculating *F. verticillioides* two days before *T. harzianum* strain 1, in one Petri plate, contact was made between the two fungi within two days of pairing. In the other two Petri plates, contact was established within four days of pairing. In all Petri plates, the pathogen was restricted to an average growth of 2.5 cm diameter, though reactions in the three Petri plates differed. In the first Petri plate, sporulation of antagonist seemed delayed till the 7th day of pairing except for the areas around point of inoculation. However in the other two Petri plates, sporulation begun by the 4th day of pairing. By the 6th day of pairing, antagonist started growing over mycelium of pathogen in all the three Petri plates. By the 9th day, mycelium of pathogen started drying up from point of contact with the antagonist backwards. Agar was not coloured and there was no zone of inhibition (Fig. 1b).

After inoculating both fungi simultaneously, antagonist grew fast, terminating growth of pathogen at an average of 3.2 cm diameter. There was no clear contact between pathogen and antagonist in any of the three plates. Some inconspicuous space (5 mm diameter on average) resembling a clear zone was observed in all Petri plates. By the 6th day, antagonist started sporulating heavily and speedily on mycelium of pathogen forming white and green knots thereupon (Fig. 1c). Agar was not coloured in any of the Petri plates while zone of inhibition was observed in some Petri plates.

T. harzianum strain 2 (IMI 380935) paired with *F. verticillioides*

After inoculating *F. verticillioides* two days after *T. harzianum* strain 2, contact was made between them within two days of pairing. Antagonist grew fast round the plates restricting pathogen to its point of inoculation on one plate and to an average of 1.3 cm diameter on the other two plates. By the 8th day of pairing, most Petri plates appeared as pure cultures of antagonist. There was no zone of inhibition and agar was not coloured (Fig. 2a).

After inoculating *F. verticillioides* two days before *T. harzianum* strain 2 however, contact was made between them within three days of pairing. Antagonist grew fast, sporulating on pathogen's mycelium, and later halting its growth at an average of 3.7 cm diameter. By the 9th day of pairing, mycelium of pathogen started drying up right from point of contact with antagonist backwards, this was not observed in

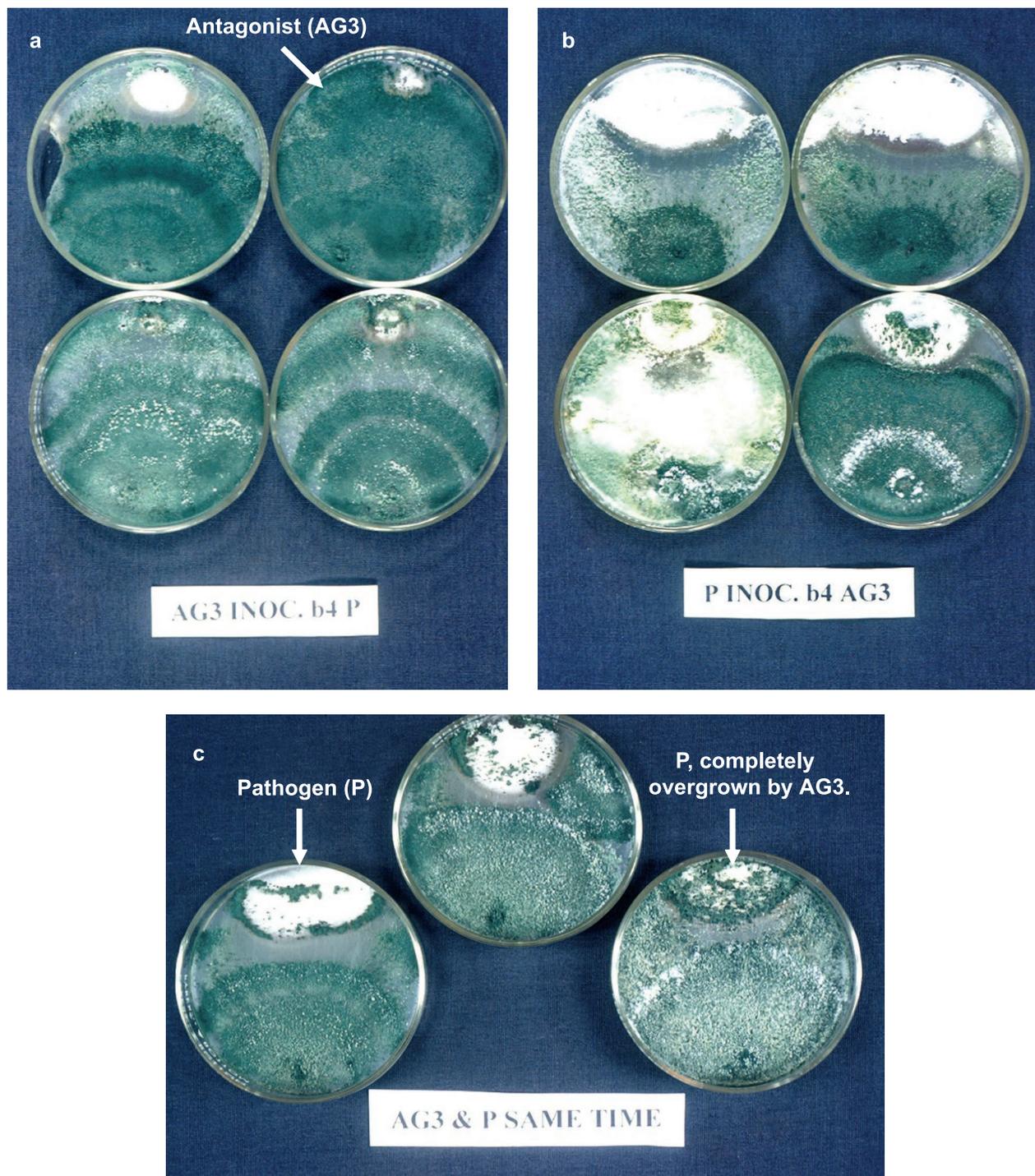


Fig. 1. *T. harzianum* strain 1 (AG3) inoculated before P (*F. verticillioides*) (a); P inoculated before AG3 (b); AG3 inoculated simultaneously with P (c). AG3 is seen obliterating mycelia mass of P after 20 days of pairing (a). AG3 gradually distorted the mycelia growth of P from point of contact (b), sporulating with time on the entire mycelia mass of P (b & c).

pure culture of pathogen. Agar was not coloured and there was no zone of inhibition in any of the plates (Fig. 2b).

Simultaneous inoculation of both fungi also showed fast growth of antagonist stopping radial growth of pathogen at an average of 2.7 cm, later sporulating upon its mycelium by the 6th day of pairing. Agar was not coloured in any of Petri plates, but a clear zone of 6.5 cm in diameter on average seemed evident in Petri plates. By the 9th day, antagonist sporulated heavily upon entire mycelium of pathogen, which in turn gradually dried up from point of contact with antagonist backwards (Fig. 2c).

In Petri plates where *T. harzianum* strains 1 and 2 completely overgrew pathogen's mycelium, mycelium of pathogen seemed to be completely 'fed' upon and the plates appeared as pure cultures of antagonists. This was observed by the 11th day of pairing. In plates where the two *Trichoderma* strains did not completely overgrow pathogen, its mycelium was so distorted in several spots that agar surface could be seen as spots within mycelium of the pathogen. These were areas where mycelium of pathogen was completely 'fed' upon to agar surface. This was observed for both strains of *T. harzianum* irrespective of pairing method.

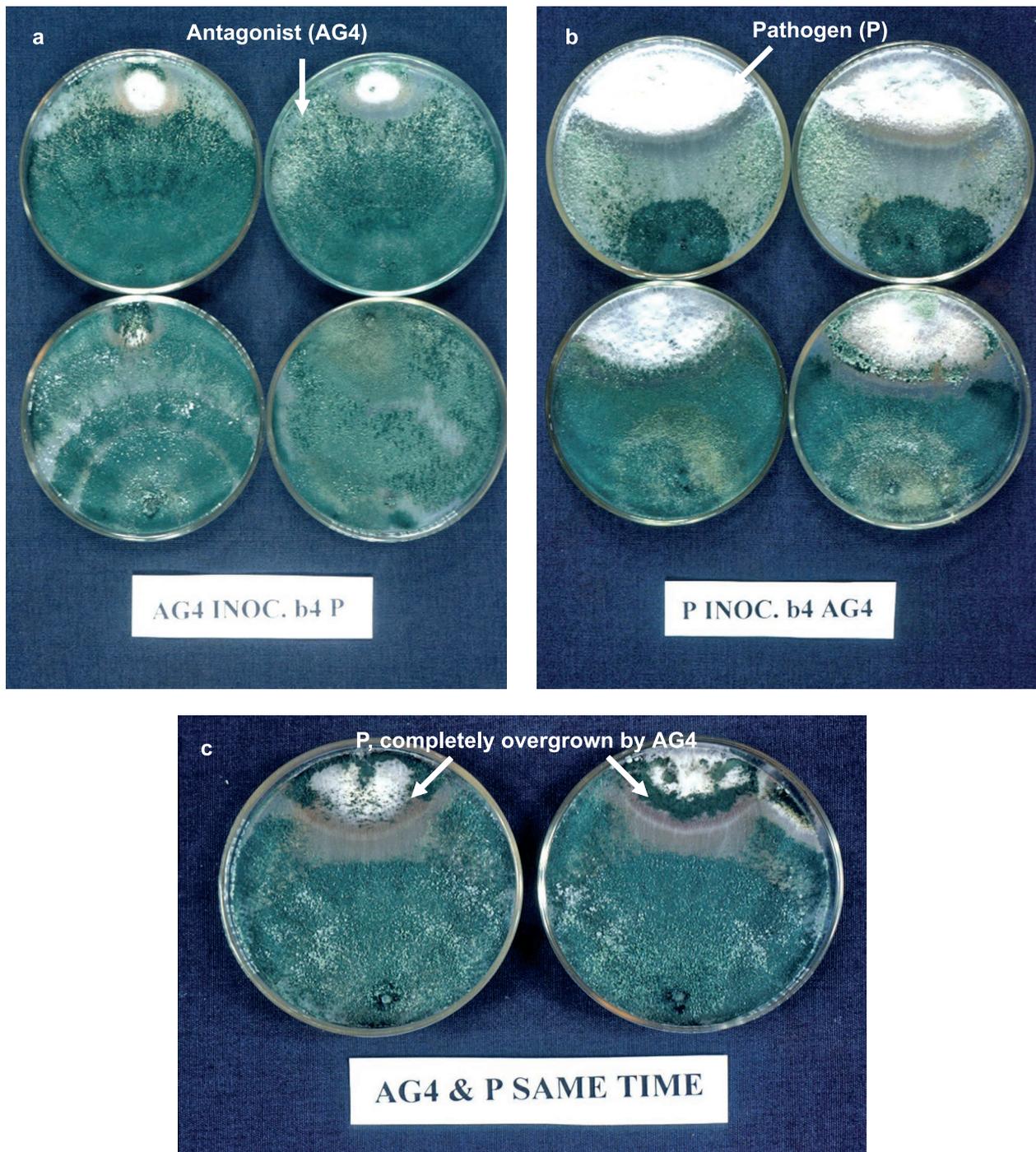


Fig. 2. *T. harzianum* strain 2 (AG4) inoculated before P (*F. verticillioides*) (a); P inoculated before AG4 (b); AG4 inoculated simultaneously with P (c). AG4 gradually grew on P until the latter was completely obliterated (a). AG4 gradually sporulated on P from point of contact until it overgrew the entire mycelia of P (b & c) after 20 days of pairing.

T. harzianum strain 3 (IMI 380938) paired with *F. verticillioides*

T. harzianum strain 3 also grew very fast on mycelium of *F. verticillioides* when inoculated two days before pathogen, stopping growth of *F. verticillioides* at an average of 0.97 cm diameter. The pathogen (*F. verticillioides*) had no chance to grow on any of Petri plates (Fig. 3a). When *F. verticillioides* was inoculated two days before *T. harzianum* strain 3, still the latter grew fast, making contact with pathogen within two days of pairing, terminating its radial growth at an average of 4.17 cm diameter (Fig. 3b).

Simultaneous inoculation of both fungi still caused antagonist growing fast, making contact with pathogen within two days of pairing, thereby stopping its further growth at an average of 2.13 cm diameter. By the 7th day of pairing, mycelium of pathogen started drying up, the occurrence that was not observed in pure culture of pathogen. By the 10th day of pairing, antagonist sporulated heavily on mycelium of pathogen in most Petri plates so that the plates almost appeared as pure cultures of antagonist (Fig. 3c).

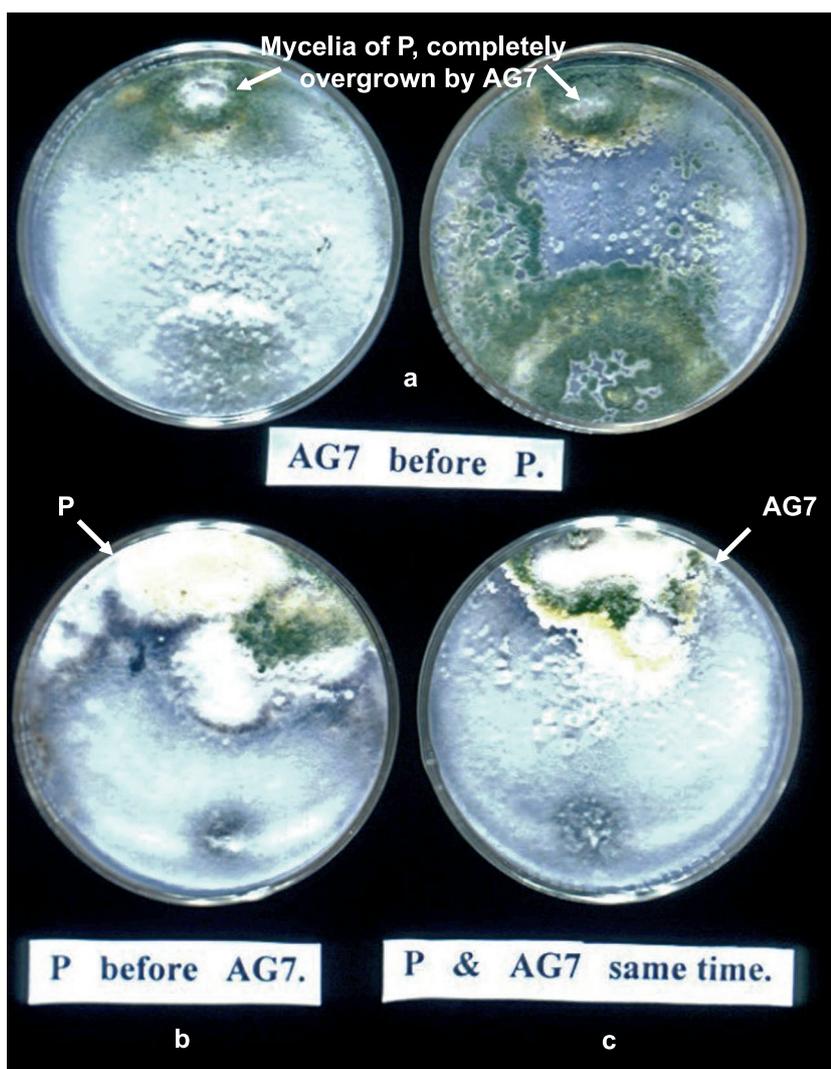


Fig. 3. *T. harzianum* strain 3 (AG7) inoculated before P (*F. verticillioides*) (a); P inoculated before AG7 (b); AG7 inoculated simultaneously with P (c). Mycelia of P is seen in 'a' completely overgrown by AG7 after 20 days of pairing.

Suppression of growth of *F. verticillioides* by the three strains of *T. harzianum* in all pairing methods

In the analysis of growth suppression of pathogen by antagonists (Table 1), means for growth suppression of *F. verticillioides* by the three strains of *T. harzianum* were significantly different from the control ($p = 0.01$). However, means for growth suppression of *F. verticillioides* by the three *T. harzianum* strains were not significantly different from each other ($R^2 = 0.96$). Results of 'inoculating antagonist before pathogen' was significantly different ($p = 0.01$) from results obtained using other two pairing methods (Table 2). However at $p = 0.05$ (Table 2), simultaneous inoculation of pathogen and antagonist was also significantly different from inoculating pathogen before antagonist. F value for the pairing methods was highly significant ($p > 0.0001$) while that for antagonist was not ($p > 0.66$). Interaction between antagonist and pairing method ($p > 0.026$) was also significant (Table 3).

Table 1. Comparison of means for radial growth suppression of *F. verticillioides* by *T. harzianum* strains in three pairing methods after 30 days of pairing

| Combination | Means for radial growth of pathogen |
|------------------------------|-------------------------------------|
| Control | 9.67 a |
| <i>T. harzianum</i> strain 2 | 3.22 b |
| <i>T. harzianum</i> strain 1 | 3.00 b |
| <i>T. harzianum</i> strain 3 | 2.89 b |
| LSD (0.01) | 1.03 |
| R^2 | 0.96 |

Means marked with different letters are significantly different from each other

Table 2. Comparison of growth suppression of *F. verticillioides* by *T. harzianum* strains among the pairing methods

| Inoculation method | Means for radial growth of pathogen | Means for radial growth of pathogen |
|---|-------------------------------------|-------------------------------------|
| | LSD (0.01) = 1.03 | LSD (0.05) = 0.76 |
| Pathogen inoculated before antagonist (Pb4AG) | 4.44 a | 4.44 a |
| Antagonist and pathogen inoculated simultaneously (AGP) | 3.44 a | 3.44 b |
| Antagonist inoculated before pathogen (AGb4P) | 1.22 b | 1.22 c |

Means marked with different letters are significantly different from each other

Table 3. ANOVA table for growth suppression of *F. verticillioides* by *T. harzianum* strains in three pairing methods

| Source | DF | MS | F-Value | P > F |
|----------------------------|----|-------|---------|----------|
| Model | 11 | 32.28 | 53.01 | 0.0001** |
| Antagonists | 2 | 0.26 | 0.43 | 0.66 |
| Pairing methods | 2 | 24.48 | 40.21 | 0.0001** |
| Antagonist* pairing method | 4 | 2.04 | 3.35 | 0.026* |
| Replicates | 2 | 0.36 | 0.59 | 0.56 |
| Error | 24 | 0.61 | | |
| Total | 35 | 10.54 | | |

where:

Antagonists – *T. harzianum* strains

Antagonist*pairing method – Interaction between antagonist and pairing method

* significant

** highly significant

Suppression of growth of *F. verticillioides* by the three strains of *T. harzianum* in separate pairing method

In ‘inoculation of antagonist before pathogen’ (AGb4P), means for growth suppression of pathogen by all the *T. harzianum* strains (Table 4) differed significantly from control ($p = 0.05$). However means for growth suppression of *F. verticillioides* by the *T. harzianum* strains were not significantly different from each other ($R^2 = 0.27$). In ‘inoculation of pathogen before antagonist’ (Pb4AG), means for growth suppression of *F. verticillioides* by the three *T. harzianum* strains were significantly different from control ($p = 0.05$, $R^2 = 0.78$). Mean for growth inhibition of pathogen by *T. harzianum* strain 1 also differed significantly from those of strains 2 and 3 which were not significantly different from each other (Table 4).

In ‘simultaneous inoculation of pathogen and antagonist’ (AGP), means for growth suppression of *F. verticillioides* by the three strains of *T. harzianum* also differed significantly from control ($p = 0.05$, $R^2 = 0.80$) (Table 4). Mean for growth suppression by *T. harzianum* strain 1 significantly differed from that of *T. harzianum* strain 3, but not from strain 2.

Table 4. Comparison of means for radial growth suppression of *F. verticillioides* by *T. harzianum* strains in separate pairing method after 30 days of pairing

| Combination | Means for radial growth of pathogen | | |
|------------------------------|-------------------------------------|--------|---------|
| | AGb4P | Pb4AG | AGP |
| Control | 9.67 a | 9.67 a | 9.67 a |
| <i>T. harzianum</i> strain 1 | 1.67 b | 3.33 b | 4.00 b |
| <i>T. harzianum</i> strain 2 | 1.33 b | 5.00 c | 3.33 bc |
| <i>T. harzianum</i> strain 3 | 0.67 b | 5.00 c | 3.00 c |
| LSD (0.05) | 3.23 | 1.51 | 0.76 |
| R2 | 0.27 | 0.78 | 0.80 |

Means marked with different letters are significantly different from each other

AGb4P – antagonist inoculated before pathogen

Pb4AG – pathogen inoculated before antagonist

AGP – antagonist inoculated simultaneously with pathogen

DISCUSSION

The ability of the three strains of *T. harzianum* to significantly inhibit growth of pathogen (*F. verticillioides*) when compared to control in the three pairing methods supported the submission of many researchers on *Trichoderma* species and their antagonistic potential against several fungi (Ahmed *et al.* 2000; Howell 2003). A major characteristic of the genus *Trichoderma*, as reported by Howell (2003) is its ability to parasitize other fungi in the course of their antagonistic exploits. The results specifically supported the submission of Ahmed *et al.* (2000) on the antagonistic potential of *T. harzianum*. Significant growth inhibition of *F. verticillioides* by the three *T. harzianum* strains over control show their antagonistic ability *in vitro* just as it was shown *in vivo* against other fungi by many researchers (Ahmed *et al.* 2000; Howell 2003; Sobowale *et al.* 2007). The fast growth of the *T. harzianum* strains in all Petri plates, irrespective of pairing method with *F. verticillioides* is indicative of their high sporulating capacity which enabled them to colonize more space on Petri plates, leaving little or sometimes no space for growth of pathogen. A fast and high rate of sporulation is a major characteristic of a good antagonist (Campbell 1988; Sharma and Sankaran 1988).

Mode of inhibition of the pathogen by any of the three *T. harzianum* strains might include competition for space and nutrients. This was because of the ability of any of them to grow fast round a Petri plate even in the presence of pathogen, irrespective of pairing method, colonizing more space and utilizing the nutrients thereof. The pathogen, in the presence of antagonists, (particularly *T. harzianum* strains 2 and 3) seemed to be depleted of essential nutrients, this being suggested by the drying up of mycelial mass of *F. verticillioides* from point of contact with the antagonists backwards, a phenomenon which was not observed in a pure culture of pathogen. Deacon and Berry (1992) concluded that competition for nutrients might be the commonest mechanism in biocontrol and that other mechanisms only serve as facilitating mechanisms. More

work however needs to be done to ascertain this as a mode of inhibition of the pathogen by *Trichoderma* strains.

Mode of growth inhibition of pathogen by *Trichoderma* strains might also include mycoparasitism. This was suggested by a heavy sporulation of the three *Trichoderma* strains upon mycelial mass of *F. verticillioides* in all Petri plates, irrespective of pairing method, where some Petri plates appeared as pure cultures of antagonists. The same was also suggested by a complete distortion and subsequent total disappearance of mycelial mass of *F. verticillioides* (which had grown initially to some points) where the antagonists overgrew them. At such points, re-isolation of *F. verticillioides* became impossible. Although this is not enough to ascertain mycoparasitism as a mode of antagonism, yet it underscores the promising antagonistic potential of any of the three *T. harzianum* strains against *F. verticillioides*. More work however is needed to be done to ascertain the mode(s) of inhibition. In the experiments of Sobowale *et al.* (2007), performance of *T. harzianum* strain 2 against *F. verticillioides* within maize (*Z. mays*) stem in the field also showed a promising antagonistic capability of this *Trichoderma* strain *in vivo*.

The agar plates which were not coloured at any culture point in the whole experiment might suggest the lack of metabolite production by the *T. harzianum* strains. However the clear zone which was observed only in 'simultaneous inoculation of pathogen and antagonist' for *T. harzianum* strains 1 and 2 might suggest a probable production of colourless metabolite by the two strains which moved ahead of them, colonizing space within the agar, thereby restricting further growth of *F. verticillioides*. Thrane *et al.* (2000) suggested that production of extracellular hydrolytic enzymes by *Trichoderma* species are important determinants of their antagonistic ability. However, this is not enough to suggest antibiosis as one of the modes of antagonism by the *T. harzianum* strains.

The lack of significance amongst the three *T. harzianum* strains in their growth inhibition of *F. verticillioides* suggests closeness in antagonistic potential of the three *Trichoderma* strains against *F. verticillioides* ($R^2 = 0.96$). Any of the three *Trichoderma* strains could then be said to be good enough to successfully inhibit growth of *F. verticillioides*. Significance of 'inoculating antagonist before pathogen' over other two pairing methods, even at $p = 0.01$ is indicative of its preference over them, for effective growth inhibition of *F. verticillioides*. This means that for effective growth inhibition of *F. verticillioides*, it is better to have any of the three *T. harzianum* strains inoculated before *F. verticillioides*. Deductively, this also suggests, that if any of the *T. harzianum* strains is tried as potential antagonists of *F. verticillioides in vivo*, it may be better for the antagonists to colonize tissues of the maize (*Z. mays*) plant ahead of *F. verticillioides* for a more effective growth suppression of the latter within maize plant. This was true for *T. harzianum* strain 2 when tried against *F. verticillioides* within maize (*Z. mays*) stem in the field (Sobowale *et al.* 2007). Significance of 'simultaneous inoculation of pathogen and antagonist' over 'inoculating pathogen before antagonist' at $p = 0.05$ suggests that it is even better for the *T. harzianum* strains to be inoculated simultaneously with *F. verticillioides* than for pathogen to be inoculated before

antagonist. This also suggests that for effective growth suppression of *F. verticillioides* within maize plant, it is better for any of the *T. harzianum* strains to occur at same time with the pathogen within tissues of the plant, than for the pathogen to occur before the antagonists.

A highly significant F value ($p > 0.0001$) for pairing method (Table 3) showed that growth inhibition of *F. verticillioides* by the *T. harzianum* strains differed among pairing methods. This means that pairing method has an effect on effectiveness of growth inhibition of *F. verticillioides* by the *T. harzianum* strains. This confirms results shown in Table 2 obtained for the different pairing methods. The time of occurrence could therefore be said to play a significant role in determining indices of antagonism by the *T. harzianum* strains. This might be connected with their mode of antagonism which might have included competition for space and nutrients.

A significant F value ($p > 0.026$) for interaction between antagonist and pairing method showed that growth inhibition of *F. verticillioides* by any particular strain of *T. harzianum* differed from one pairing method to the other. For instance, *T. harzianum* strain 1 differed significantly from strains 2 and 3 in its growth inhibition of *F. verticillioides* when pathogen was inoculated before antagonist (Table 4). It was however not significantly different in the other two pairing methods (Tables 5, 7). Growth inhibition of *F. verticillioides* by the *T. harzianum* strains could thus be said to be different among and within pairing methods. Significance of *T. harzianum* strain 1 over strains 2 and 3 is suggestive, particularly if the three of them are to be tried as potential antagonists of *F. verticillioides* within maize plant (Table 4).

In conclusion, it can be said that *T. harzianum* strain 1, with some more research, could address the problem of endophytic *F. verticillioides* within maize plant. The three *Trichoderma* strains might ultimately succeed as antagonists of *F. verticillioides in vitro* and *in vivo*, with some more research. If this is achieved, either by strain 1 or any of the other two strains of *T. harzianum*, incidence of fumonisins within maize seeds could also be a secondary target.

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POLISH SUMMARY

OGRANICZENIE WZROSTU *FUSARIUM VERTICILLIOIDES* NIREN. PRZY WYKORZYSTANIU SZCZEPÓW *TRICHODERMA HARZIANUM* Z CZĘŚCI ROŚLIN KUKURYDZY (*ZEA MAYS*) I ICH RIZOSFERY

Porównano trzy szczepy *Trichoderma harzianum* (szczep 1: IMI 380934; szczep 2: IMI 380935 i szczep 3: IMI 380938) pod względem ich zdolności ograniczania odśrodkowego wzrostu *Fusarium verticillioides in vitro*. Każdy szczep *Trichoderma* z zaszczepiono na płytki Petriego o średnicy 9 cm, z zakwaszoną pożywką agarowo-ziemniaczną, stosując 3 metody. Stosując Procedurę GLM SAS uzyskano zróżnicowane ograniczenie wzrostu patogena przez szczepy *Trichoderma*. Inhibicja wzrostu przez każdy przez każdy ze szczepów *T. harzianum* różniła się istotnie od kontroli, niezależnie od wykorzystanej metody ($p = 0,01$, $R^2 = 0,96$). Wyższą inhibicję *F. verticillioides* uzyskano w przypadku wcześniejszego zaszczepienia antagonisty niż patogena, nawet przy $p = 0,01$. Mechanizm ograniczenia wzrostu obejmował mykopasożytnictwo oraz konkurencję o miejsce i składniki pokarmowe. Inhibicja wzrostu patogena była różna pomiędzy ($p > 0,0001$) i w ramach ($p > 0,026$) zastosowanych metod. *T. harzianum* szczep 1 lepiej ograniczał wzrost patogena niż dwa pozostałe szczepy wtedy, gdy był zaszczepiony wcześniej niż patogen, podczas gdy *T. harzianum* szczep 3 okazał się lepszy, gdy patogena i antagonistę zaszczepiono jednocześnie ($p = 0,005$). Wobec powyższego różne szczepy *T. harzianum* mogą być wykorzystywane jako obiecujący antagoniści grzyba *F. verticillioides*.