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GROWTH INHIBITION OF *FUSARIUM VERTICILLIOIDES* (SACC.) NIRENBERG BY ISOLATES OF *TRICHODERMA PSEUDOKONINGII* STRAINS FROM MAIZE PLANT PARTS AND ITS RHIZOSPHERE

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Abstract: Ability of five strains of *Trichoderma pseudokoningii* (antagonists) to suppress radial growth of *Fusarium verticillioides* (Sacc.) Nirenberg (=*Fusarium moniliforme* Sheldon) was examined *in vitro*. These were *T. pseudokoningii* strain 1 (IMI 380933), strain 2 (IMI 380937), strain 3 (IMI 3809 39), strain 4 (IMI 380940) and strain 5 (IMI 380941). Each strain was paired with pathogen by inoculating at opposite ends of 9 cm petri plates using three pairing methods. Gradings were assigned to varied growth inhibition of pathogen by antagonists and analysed using GLM procedure (SAS). Growth suppression of *F. verticillioides* by all strains of *T. pseudokoningii* was significantly different (R^2=0.98, p=0.05) from control in all pairing methods. It differed significantly (p>0.0003) among the strains in all pairing methods. Growth suppression also differed significantly among (p>0.0001) and within (p>0.018) pairing methods. Growth suppression was best when antagonists were inoculated before pathogen. Suppression mechanisms include mycoparasitism and competition for space and nutrients. *T. pseudokoningii* strains 3 and 4 had the best (p=0.05) growth suppression of *F. verticillioides* and could be used as biocontrol agents for endophytic *F. verticillioides* in maize plant. This experiment was conducted in the search for resident microorganisms that might be capable of checking *F. verticillioides* within maize plant by competitive exclusion in subsequent experiments.

Key words: *Trichoderma pseudokoningii*, *Fusarium verticillioides*, antagonist, pathogen, persistence, internodes

INTRODUCTION

Maize is reported to be of worldwide distribution with lower price and wider range of uses than other cereals. It is the basic staple food in a number of developing countries (Bunting et al. 1978). There are many pathogens including *Fusarium verticillioides* that threaten its global production (MacDonald and Chapman 1996). *F. verticillioides* is an important pathogen causing diseases on the root, stalk and ear of maize (McKeen 1953; Visconti and Doko 1994). It is the most common single species of fungus in samples of maize obtained from Central America, Africa, and Asia between 1992–1995 (Roane 1950; MacDonald and Chapman 1996). It poses severe threat to human and animal health due to its potent mycotoxigenic and carcinogenic characteristics (Marasas 1988; Julian et al. 1995). Munkvold and Carlton (1996) confirmed its ability to move systemically from seed to stalk and then to kernels in maize plants.

Trichoderma species have been commonly used as biocontrol fungi against many pathogens both *in vitro* and *in vivo* (Paavanen-Huhtala et al. 2000). Recognition of potential of *Trichoderma* species as biocontrol agents of plant diseases dated back to 1930s (Weindling 1932). In subsequent years, control of many diseases using *Trichoderma* species has been reported. Ability to parasitize other fungi remains a major characteristic of the group (Howell 2003). Ahmed et al. (2000) used *T. harzianum* to induce systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici*. Their result indicated that seed treatments with *T. harzianum* spores significantly reduced stem necrosis. In experiments conducted by Yates et al. (2000), *Trichoderma viride* isolated from roots of corn plants was able to suppress radial colony extension of *F. verticillioides* on agar as well as fumonisin B_1 (FB_1) on corn kernels. They concluded that *T. viride* might be useful in biological control as a pre-harvest agent to prevent disease during plant development and/or as a post-harvest agent to suppress FB_1 production during seed storage.

Different antagonists have different mechanisms by which they accomplish process of antagonism, these include among others antibiosis, mycoparasitism, and competition (Campbell 1988; Wells 1988). *In vitro*, cell-free metabolites of *Trichoderma virens* DAR74290 completely inhibited growth of *Phytophthora erythroseptica* (Etebarian et al. 2000). In the soil, fungi exhibiting antagonism by antibiosis may not be too effective in the soil but mycoparasites are more effective. This is because they possess some attributes that increase their potential as biocontrol agents (Sharma and Sankaran 1988). Competition for nutrients however is the most common mechanism in biocontrol and other mechanisms only serve as facilitating mechanisms (Deacon and Berry 1992).

A good antagonist should allow a simple and inexpensive multiplication in the laboratory, and it should also have a fast and high rate of sporulation (Kerr 1982; Campbell 1988; Sharma and Sankaran 1988; Hayes 1992). The objectives of the study were to isolate *F. verticillioides* and *Trichoderma* species from different parts of the maize plant and its rhizosphere, to determine fungi that would suppress radial growth of pathogen (*F. verticillioides*) *in vitro* and to evaluate the effect of pairing methods on effective antagonism *in vitro*.

MATERIALS AND METHODS

Isolation and identification of pathogen (*F. verticillioides*)

F. verticillioides was isolated from naturally infected maize stem by stalk sectioning. Infected stems were brought to the laboratory from the field and split open longitudinally with sharp sterile knife. Several rotted tissues from the infected parts were cut and surface sterilized by soaking for 5 minutes in 1% sodium hypochlorite before rinsing in five changes of sterile distilled water. They were picked onto sterile filter paper using sterile forceps and wrapped with filter paper for 3–5 minutes to allow drying. The cut sections were later picked onto several sterile prepared plates of 15 ml Acidified Potato Dextrose Agar (APDA) and incubated for 8–10 days at 28–30°C. Mixed cultures of *Fusarium* species. were subcultured into separate plates and incubated again to obtain pure cultures. Separate *Fusarium* species were cultured on Potassium Chloride (KCl) medium to aid identification of *F. verticillioides*, which forms long chains of conidia on KCl medium (Nelson et al. 1983).

Isolation and identification of strains of *T. pseudokoningii* (antagonists)

Five methods were used to isolate the *Trichoderma* species from different parts of maize plant and its rhizosphere viz., stalk sectioning, soil plate method (Warcup 1950), soil dilution plate method (Tuite 1969), soil washings (Dhingra and Sinclair 1985) and modified methods of Janisiewicz (1988) and Roberts (1990). Pure cultures were stored on silica gel using the method of Smith and Onions (1983). They were sent to International Mycological Institute (IMI) England for identification.

Pairing of pathogen and antagonists

Each fungus (AGx) was paired with the pathogen (P) as a potential antagonist against the latter. Pathogen and antagonist were inoculated at opposite ends of 9cm petri plate of APDA. Three pairing methods were employed, viz., (a) antagonist inoculated two days earlier than pathogen (AGxb4P); (b) pathogen inoculated two days erlier than antagonist (Pb4AGx); and (c) pathogen and antagonist inoculated simultaneously (AGxP). Experiments were done in three replications and all plates were incubated at 28–30°C for 30 days.

Data collection

Average measurements of growth diameter of pathogen and antagonist were taken daily with a caliberated ruler until growth stopped. Rating scale of 0 to 10 assigned to different degrees of inhibition of pathogen's radial extension were recorded in the 9 cm diameter petri plates of which was divided into 10 equal parts, (i.e multiples of 0.9 cm), viz., 0: if antagonist grows and covers the whole 9 cm plate; 1: if pathogen covers between 0 to 0.90 cm of plate diameter; 2: if pathogen covers between 0.91 to 1.80 cm of plate diameter; 3: if pathogen covers between 1.81 to 2.70 cm of plate diameter; 4: if pathogen covers between 2.71 to 3.60 cm of plate diameter; 5: if pathogen covers between 3.61 to 4.50 cm of plate diameter; 6: if pathogen covers between 4.51 to 5.40 cm of plate diameter; 7: if pathogen covers between 5.41 to 6.30 cm of plate diameter; 8: if pathogen covers between 6.31 to 7.20 cm of plate diameter; 9: if pathogen covers between 7.21 to 8.10 cm of plate diameter; and 10: if pathogen grows between 8.1 cm and the whole 9 cm diameter plate.

Statistical Analysis

Rating results in each set of triplicate were subjected to analysis of variance (ANOVA) using the General Linear Model option of SAS. This was done to determine comparative effectiveness of *T. pseudokoningii* strains as antagonist(s) against the pathogen (*F. verticillioides*) *in vitro* and the particular pairing method assured to determine effective inhibition of pathogen.

RESULTS

Trichoderma pseudokoningii strain 1 (IMI 380933) paired with *F. verticillioides*

When *T. pseudokoningii* strain 1 was inoculated earlier than the pathogen (*F. verticillioides*), the antagonist grew rapidly stopping growth of pathogen at an average of 1.7 cm diameter by the 4th day of pairing. By the 5th day, antagonist began to grow on top of pathogen, completely overgrowing it by the 11th day of pairing and all plates looked like pure cultures of antagonist. When *F. verticillioides* was inoculated earlier than *T. pseudokoningii* strain 1, antagonist grew and terminated growth of pathogen at an average of 3.3 cm diameter by the 4th day. The antagonist continued growing over hyphae of pathogen by the day. When both organisms were inoculated simultaneously, antagonist grew and restricted pathogen to an average growth of 2.5 cm diameter by the 4th day of pairing. After ten days, antagonist began to grow in spots all over hyphae of pathogen (Fig. 2).

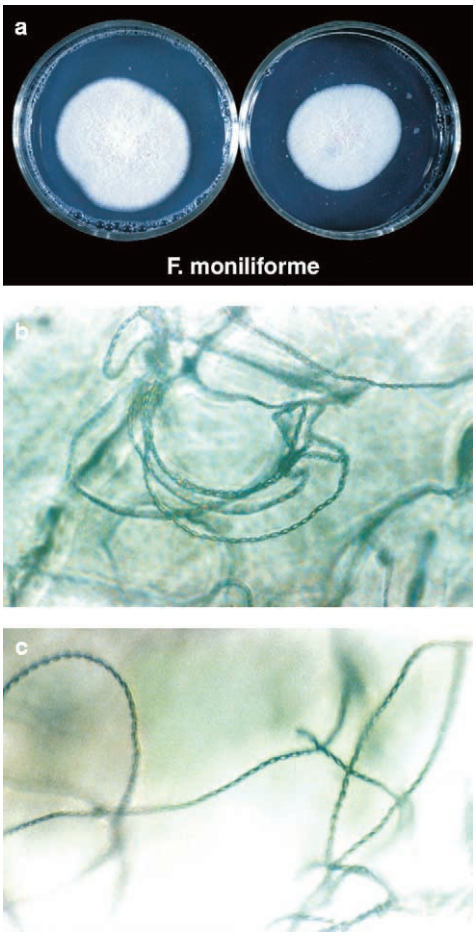

Fig. 1. a: *Fusarium verticillioides* on PDA, b: chains of *F. verticillioides*, c: long chains of microconidia

In all pairing methods, after contact was made between pathogen and antagonist, *F. verticillioides* began to produce a yellow-green metabolite into the agar, but there was no clear zone of inhibition. Despite daily growth and sporulation of antagonist on pathogen, the metabolite production increased daily within the agar beneath hyphae of antagonist. By the 17th day of pairing, the metabolite had covered 4.5 cm of the whole 9 cm plate when viewed from the reverse side. Production of metabolite was more intense when antagonist was inoculated earlier than pathogen as

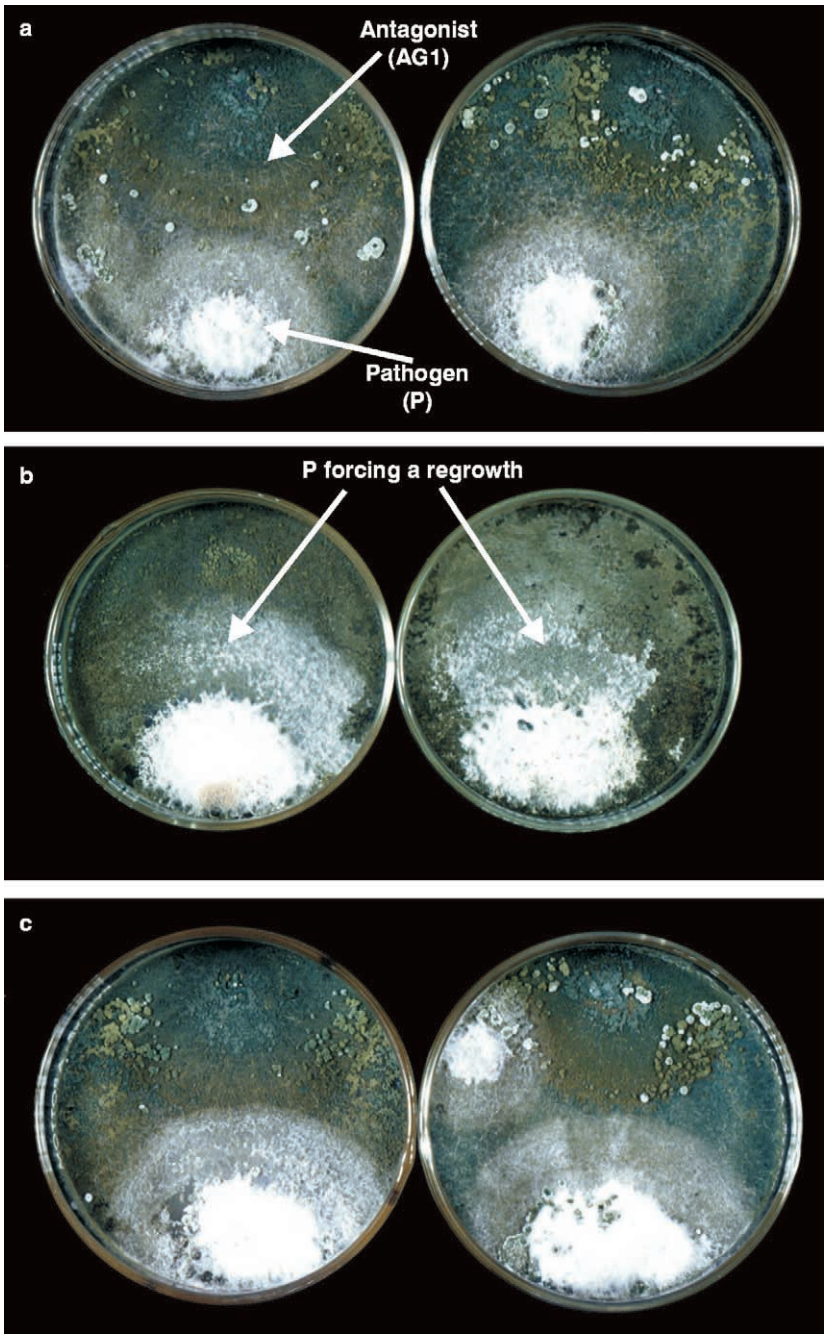

Fig. 2. a: *Trichoderma pseudokoningii* str. 1 (AG1) inoculated prior to *Fusarium. verticillioides* (P), b: *F. verticillioides* inoculated before *T. pseudokoningii* str. 1, c: *T. pseudokoningii* str. 1 inoculated simultaneously with *F. verticillioides*. P is seen trying to force a regrowth through AG1 in regions already covered by the latter (AG1) after 20 days of pairing

compared to other two pairing methods. It was also more intense when antagonist and pathogen were inoculated simultaneously than when pathogen was inoculated earlier. There was no metabolite production in pure culture of pathogen (control, Fig. 1). Hyphae of antagonist growing in the region of metabolite production began to reduce spore production with time and new hyphae of pathogen started to grow on already grown hyphae of antagonist in the region of metabolite (Fig. 2). However, by the 20th day of pairing, new hyphal mass regrowth of antagonist began on new hyphae of pathogen, completely distorting them. By the 26th day, production of metabolite by pathogen ceased at an average of 5.2 cm diameter and antagonist regrew and resumed sporulation on the entire fresh hyphal mass of pathogen.

Trichoderma pseudokoningii* strain 2 (IMI 380937) paired with *F. verticillioides

When *T. pseudokoningii* strain 2 was inoculated prior to *F. verticillioides*, antagonist grew fast, restricting growth of pathogen to an average of 1.5 cm diameter by the 4th day of pairing. By the 8th day, antagonist grew over mycelia of pathogen, gradually distorting it at the base. When *F. verticillioides* was inoculated earlier than *T. pseudokoningii* strain 2, antagonist grew fast making contact with pathogen two days after pairing, restricting its growth to an average of 2.5 cm diameter by the 4th day. When both organisms were inoculated simultaneously, antagonist grew and terminated growth of pathogen at an average of 2.2 cm diameter by the 4th day. By the 8th day, antagonist started to sporulate on mycelial mass of pathogen, growing deep into it at the base (Fig. 3).

Trichoderma pseudokoningii* strain 3 (IMI 380939) paired with *F. verticillioides

When *T. pseudokoningii* strain 3 was inoculated earlier than *F. verticillioides*, antagonist grew rapidly round the plates within three days, leaving little space (0.5 cm diameter) for growth of pathogen. By the 8th day, most petri plates appeared as pure cultures of antagonist (Fig. 4). When *F. verticillioides* was inoculated earlier than *T. pseudokoningii* strain 3, antagonist grew and stopped growth of pathogen at an average of 2.6 cm diameter by the 3rd day of pairing. By the 14th day of pairing, antagonist completely overgrew mycelia of pathogen (Fig. 4). When both organisms were inoculated simultaneously, antagonist grew and terminated growth of pathogen at an average of 2.2 cm diameter by the 3rd day, gradually growing upon the hyphae of pathogen, completely overgrowing it by the 8th day in some petri plates (Fig. 4). In all pairing methods, contact was made within two days of pairing and mycelia of pathogen dried up faster than in pure culture.

Trichoderma pseudokoningii* strain 4 (IMI 380940) paired with *F. verticillioides

When *T. pseudokoningii* strain 4 was inoculated earlier than *F. verticillioides*, antagonist grew fast, terminating growth of pathogen at an average of 1.2 cm diameter by the 3rd day. When *F. verticillioides* was inoculated earlier than *T. pseudokoningii* strain 4, antagonist grew and stopped growth of pathogen at an average of 2.5 cm diameter by the 3rd day. When both organisms were inoculated simultaneously, antagonist grew and terminated growth of pathogen at an average of 2.1 cm diameter also by the 3rd day of pairing. In all pairing methods, contact was made within two days of pairing and by the 9th day, antagonist completely overgrew mycelia of pathogen

so that most petri plates looked like pure cultures of antagonist (Fig. 5). Mycelia of pathogen dried up faster than in pure culture.

Trichoderma pseudokoningii* strain 5 (IMI 380941) paired with *F. verticillioides

When *T. pseudokoningii* strain 5 was inoculated before *F. verticillioides*, antagonist grew fast restricting growth of pathogen to an average of 1.6 cm diameter by the 3rd day of pairing. When pathogen was inoculated earlier than antagonist, antagonist grew and stopped growth of pathogen at an average of 3.0 cm diameter by the 3rd day. When both organisms were inoculated simultaneously, antagonist grew and terminated growth of pathogen at an average of 2.3 cm diameter by the 3rd day. By

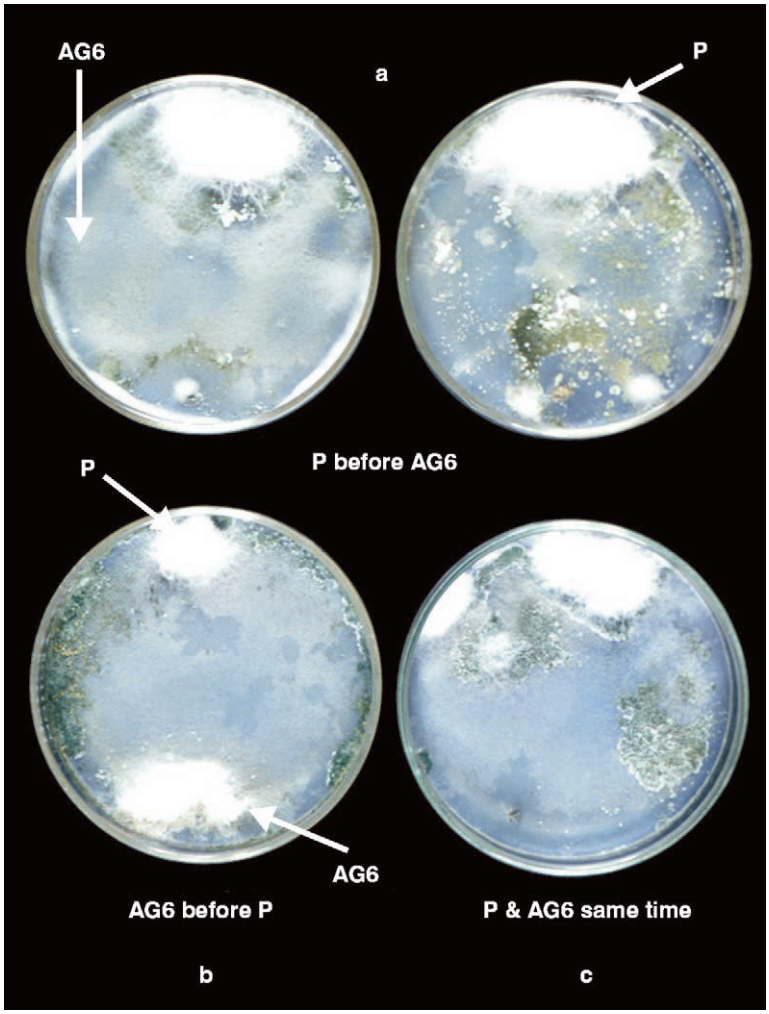

Fig. 3. a: *F. verticillioides* (P) inoculated prior to *T. pseudokoningii* str. 2 (AG6), b: AG6 inoculated prior to P, c: AG6 inoculated simultaneously with P. P is seen restricted by AG6 to small regions around inoculation points in all plates after 20 days of pairing

the 8th day, antagonist overgrew mycelia of pathogen, distorting its mycelial mass from the base. In all pairing methods, contact was made two days after pairing and mycelial mass of pathogen began to dry up after eight days of pairing (Fig. 6). This did not occur in pure culture of pathogen.

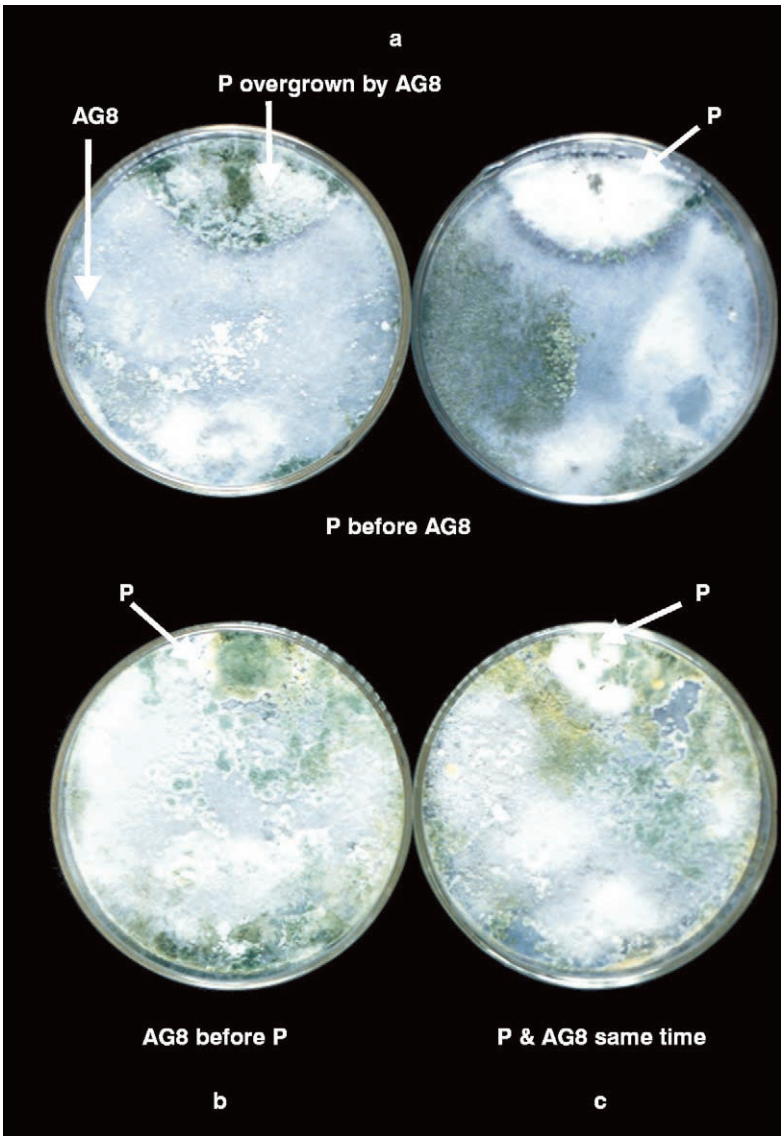

Fig. 4. a: *F. verticillioides* (P) inoculated prior to *T. pseudokoningii* str. 3 (AG8), b: AG8 inoculated prior to P, c: AG8 inoculated simultaneously with P. P is seen partly overgrown by AG8 in 'c' but completely overgrown by AG8 in 'b' and in the left petri plate of 'a' after 20 days pairing

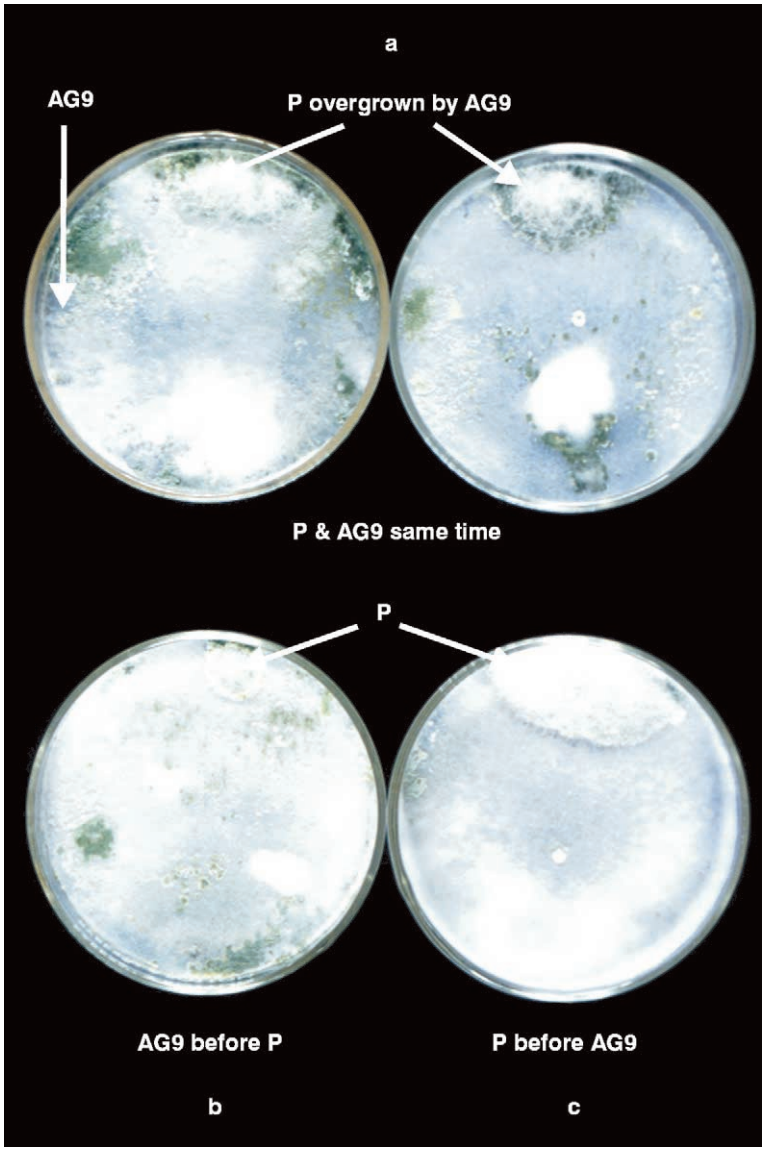

Fig. 5. a: *T. pseudokoningii* str. 4 (AG9) inoculated simultaneously with *F. verticillioides* (P), b: AG9 inoculated prior to P, c: P inoculated prior to AG9. P is seen completely overgrown by AG9 (a & b) after 20 days of pairing

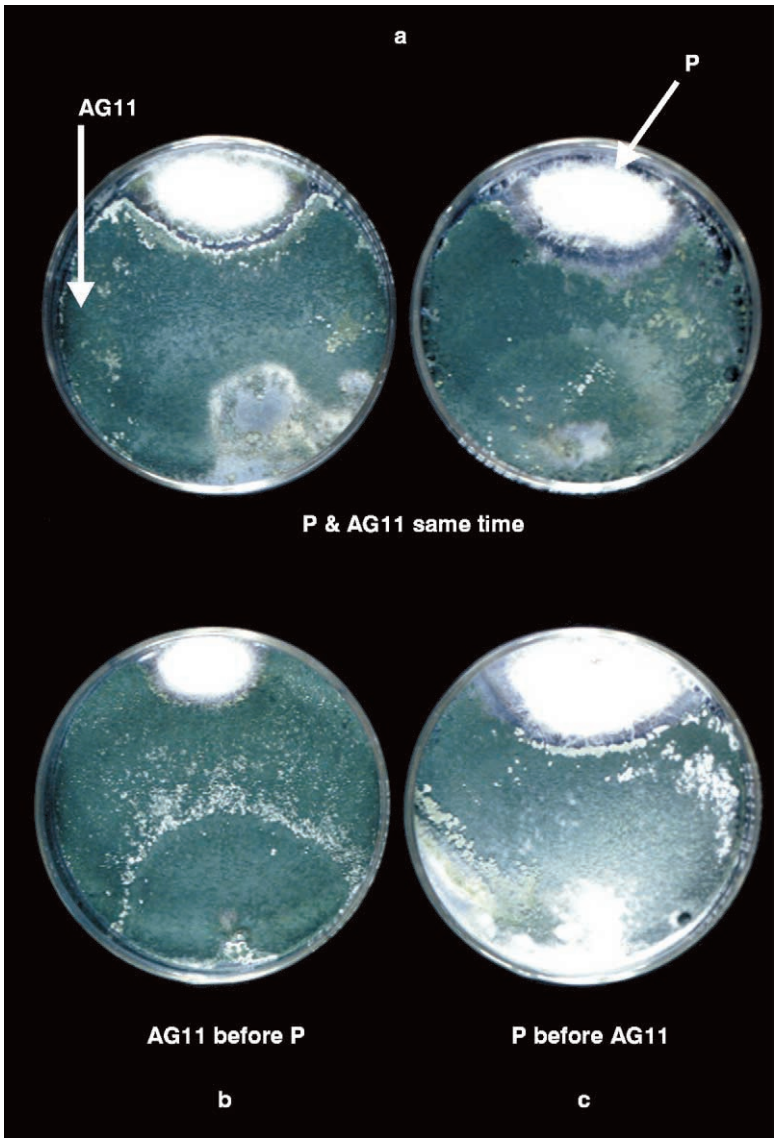

Fig. 6. a: *T. pseudokoningii* str. 5 (AG11) inoculated simultaneously with *T. verticillioides* (P), b: AG11 inoculated prior to P, c: P inoculated prior to AG11. Growth of P is seen restricted to small regions by AG11 in all petri plates after 20 days of pairing

Growth inhibition of *F. verticillioides* by all strains of *T. pseudokoningii* in separate pairing methods

Tables 1 to 4 show summary of analysis of ratings for growth inhibition of *F. verticillioides* by the five strains of *T. pseudokoningii* in each pairing method. When antagonist was inoculated earlier than pathogen (AGxb4P), means for growth inhibition of *F. verticillioides* by the 5 strains of *T. pseudokoningii* were all significantly different (R^2 = 0.75, p=0.05) from control. Mean for growth inhibition by *T. pseudokoningii* strain 3 was significantly different from those of other strains, which were not significantly different from each other (Table 1). F value (Table 4) for the strains was also signifcant (p>0.0294).

When pathogen was inoculated earlier than antagonist (Pb4AGx), means for growth inhibition of *F. verticillioides* by the 5 strains of *T. pseudokoningii* were significantly different (R^2 = 0.86, p=0.05) from control. Means for growth inhibition by *T. pseudokoningii* strains 2, 3 and 4 were significantly different from those of strains 1 and 5 (Table 2). F value (Table 4) for all strains was highly significant (p>0.0025).

When antagonist and pathogen were simultaneously inoculated (AGxP), means for growth inhibition of *F. verticillioides* by the 5 strains of *T. pseudokoningii* were significantly different from

Table 1. Comparison of means of radial growth inhibition of *F. verticillioides* by strains *T. pseudokoningii* when antagonist was inoculated prior to pathogen

Antagonists	Means of gradings for radial growth of pathogen
Control	9.67 a
Trichoderma pseudokoningii str. 1	2.33 b
Trichoderma pseudokoningii str. 5	2.00 b
Trichoderma pseudokoningii str. 2	2.00 b
Trichoderma pseudokoningii str. 4	2.00 b
Trichoderma pseudokoningii str. 3	0.67 c
LSD 0.05	0.97
R2	0.75

Means with different letters are significantly different from each other

Table 2. Comparison of means of radial growth inhibition of *F. verticillioides* by strains *T. pseudokoningii* when pathogen was inoculated prior to antagonist

Antagonists	Means of gradings for radial growth of pathogen
Control	9.67 a
Trichoderma pseudokoningii str. 1	4.33 b
Trichoderma pseudokoningii str. 5	4.00 b
Trichoderma pseudokoningii str. 2	3.33 c
Trichoderma pseudokoningii str. 4	3.00 c
Trichoderma pseudokoningii str. 3	3.00 c
LSD 0.05	0.60
R2	0.86

Means with different letters are significantly different from each other

Table 3. Comparison of means of radial growth inhibition of *F. verticillioides* by strains *T. pseudokoningii* when antagonist and pathogen were inoculated simultaneously

Antagonists	Means of gradings for radial growth of pathogen
Control	9.67 a
Trichoderma pseudokoningii str. 1	3.00 b
Trichoderma pseudokoningii str. 5	3.00 b
Trichoderma pseudokoningii str. 2	3.00 b
Trichoderma pseudokoningii str. 3	3.00 b
Trichoderma pseudokoningii str. 4	2.67 b
LSD 0.05	0.49
R2	0.43

Means with different letters are significantly different from each other

Table 4. Anova table for growth inhibition of *F. verticillioides* by five strains of *T. pseudokoningii* in separate pairing method

Pairing method	SV	DF	MS	F-Value	Pr > F
AGxb4P	Model	6	1.04	3.92	0.0397*
	AGx	4	1.27	4.75	0.0294*
Pb4AGx	Model	6	0.82	8.22	0.0045**
	AGx	4	1.10	11.00	0.0025**
AGxP	Model	6	0.07	1.00	0.4852
	AGx	4	0.07	1.00	0.4609

Where:
AGx = Antagonists (strains of *T. pseudokoningii*)
AGxb4P = Antagonist inoculated earlier than pathogen
Pb4AGx = Pathogen inoculated earlier than antagonist
AGxP = Antagonist and pathogen inoculated simultaneously
* = Significant
** = Highly significant

control (Table 3). However, suppression of growth of pathogen by the five strains was not significantly different from each other (R^2 = 0.43, p=0.05). F value (Table 4) for all strains was also not significant (p>0.4609).

Growth inhibition of *F. verticillioides* by all strains of *T. pseudokoningii* in the three pairing methods

Tables 5 to 7 show summary of overall analysis of ratings for growth inhibition *of F. verticillioides* by the five strains of *T. pseudokoningii* in all pairing methods. Means for growth inhibition of *F. verticillioides* by all strains of *T. pseudokoningii* were significantly different (p=0.05) from control (Table 5). Mean for growth inhibition by *T. pseudokoningii* strain 3 was significantly different from those of strains 2, 5 and 1 (R^2=0.98), but not from that of strain 4. Mean for growth inhibition of the patho-

Table 5. Comparison of means of radial growth inhibition of *F. verticillioides* by strains *T. pseudokoningii* after 30 days of pairing in all pairing methods

Antagonists	Means of gradings for radial growth of pathogen
Control	9.67 a
Trichoderma pseudokoningii str. 1	3.22 b
Trichoderma pseudokoningii str. 5	3.00 bc
Trichoderma pseudokoningii str. 2	2.78 cd
Trichoderma pseudokoningii str. 4	2.56 de
Trichoderma pseudokoningii str. 3	2.22 e
LSD 0.05	0.42
R^2	0.98

Means with different letters are significantly different from each other

Table 6. Comparison of means for growth inhibtion of *F. verticillioides* among the three pairing methods

Treatments	Means of gradings for radial growth of pathogen
Pb4AGx	3.83 a
AGxP	3.24 b
AGxb4P	2.10 c
LSD 0.05	0.22

Means with different letters are significantly different from each other
Where: Pb4AGx: Pathogen inoculated earlier than antagonist, AGxP: Antagonist and pathogen inoculated simultaneously, AGxb4P: Antagonist inoculated earlier than pathogen

Table 7. Anova table for growth inhibition of *F. verticillioides* by five strains of *T. pseudokoningii* in all pairing methods

SV	DF	MS	F-Value	Pr > F
Model	17	23.03	117.19	0.0001**
AGx	4	1.36	6.9	0.0003**
Pm	2	11.62	59.15	0.0001**
AGx*Pm	8	0.54	2.74	0.018*
Rep	2	0.13	0.66	0.52
Error	36	0.2		
Total	53	7.52		

Where:
AGx = Antagonists (strains of *T. pseudokoningii*)
Pm = Pairing methods
AGx * Pm = Interaction between antagonists and pairing methods
* = Significant
** = Highly significant

gen by *T. pseudokoningii* strain 4 was significantly different from those of strains 5 and 1 but not significantly different from that of strain 2. Mean for growth inhibition by *T. pseudokoningii* strain 2 was significantly different from that of strain 1 but not significantly different from that of strain 5. However, suppression of radial extension of *F. verticillioides* by strains 5 and 1 was not significantly different from each other (Table 5).

The method of inoculating antagonist earlier than pathogen was significantly different ($p=0.05$) from the other two pairing methods (Table 6). Simultaneous inoculation of antagonist and pathogen was also significantly different from inoculating pathogen prior to antagonist. F values for all strains of *T. pseudokoningii* and the pairing methods (Table 7) were highly significant ($p>0.0003$ and $p>0.0001$ respectively). Interaction between antagonists and pairing methods was also significant ($p>0.018$).

DISCUSSION

Significance of radial growth suppression of *F. verticillioides* by all strains of *T. pseudokoningii* over control in all pairing methods showed the antagonistic potential of genus *Trichoderma* against pathogens as concluded by Paavanen-Huhtala et al. (2000) and Etebarian et al. (2000). *Trichoderma* species have been consistently reported over the years to have successfully controlled many diseases of plants (Howell 2003). Fast growth and high sporulating capacity (Sharma and Sankaran 1988) of all the *T. pseudokoningii* strains aided their aggressiveness against *F. verticillioides*. These properties enhanced their ability to compete for space, leaving little space for growth of pathogen. Rapid drying rate of F. *verticillioides* when paired in culture with *T. pseudokoningii* strains than when in pure culture suggests competition for space and nutrients as part of mechanism for growth inhibition. It showed that *F. verticillioides* was obviously depleted of nutrients in paired culture with any of *T. pseudokoningii* strains. Deacon and Berry (1992) and Howell (2003) reported competition for space and nutrients as a more general mechanism in

biocontrol. Sporulation *of T. pseudokoningii* strains on mycelial mass of *F. verticillioides* and distortion of mycelial growth suggested mycoparasitism as part of mechanism for growth suppression (Sharma and Sankaran 1988). Several workers reported many and varied mechanisms for biological control within the genus *Trichoderma* (Ahmed et al. 2000; Howell 2003).

The metablite production by *F. verticillioides* could be a response to pathogen in paired culture in trying to check *T. pseudokoningii* strain 1. This may be true as there was no metabolite production in pure culture of *F. verticillioides* and as production was more intense when antagonist was inoculated earlier than pathogen. Temporary reduction in sporulation of *T. pseudokoningii* strain 1 in region of metabolite production and growth of new hyphae of pathogen on antagonist suggested that the metabolite could have exerted a temporarily harmful effect on antagonist. However, growth of new hyphae of *T. pseudokoningii* strain 1 on the newly growing hyphae of *F. verticillioides* and the eventual cessation of metabolite production by the latter may indicate a better competitive ability of antagonist even in the presence of a 'supposedly harmful' metabolite produced by pathogen. The sporulation and eventual distortion of a new hyphal mass of pathogen by antagonist also indicated mycoparasitic capability of *T. pseudokoningii* strain 1 in the presence of harmful excretions by *F. verticillioides*.

Significant interaction between antagonists and pairing methods ($p>0.018$) showed that growth inhibition of *F. verticillioides* by any particular strain *of T. pseudokoningii* differed from one pairing method to the other. This was evident in the case *of T. pseudokoningii* strain 3, which was significantly different from other strains in growth inhibition of pathogen in one pairing method (i.e inoculating antagonist earlier than pathogen), yet not significantly different from them in another pairing method (i.e inoculating antagonist and pathogen simultaneously). However, significant growth inhibition of *F. verticillioides* by *T. pseudokoningii* strains 2, 3 and 4 over strains 1 and 5 in one pairing method (inoculating pathogen earlier than antagonist) showed the preference of strains 2, 3 and 4 over strains 1 and 5. As growth inhibition of pathogen by *T. pseudokoningii* strains 3 and 4 were generally not significantly different from each other, both strains had the best radial growth suppression of *F. verticillioides*. However, *T. pseudokoningii* strain 3 could be said to be better than *T. pseudokoningii* strain 4 in growth suppression of the pathogen. This is shown by its significant growth inhibition of the pathogen over that caused by other strains in inoculation of antagonist earlier than pathogen.

Significant F values for the 5 strains of *T. pseudokoningii* in two pairing methods (inoculating antagonist earlier than pathogen and inoculating pathogen earlier than antagonist) showed that rate of radial growth suppression of *F. verticillioides* differed among the strains within each pairing method. Non-significant F value in the third pairing method (simultaneous inoculation of antagonist and pathogen) showed that there was no significant difference among the 5 strains in their growth inhibition rate of pathogen in this pairing method. This means that the overall highly significant F value for antagonists ($p>0.0003$) was contributed only by their significance in two of the three pairing methods (i.e inoculating antagonist earlier than pathogen and inoculating pathogen earlier than antagonist). Highly signifi-

cant F value ($p>0.0025$) for antagonists obtained when pathogen was inoculated earlier than antagonist showed that difference among strains of *T. pseudokoningii* in inhibiting growth of *F. verticillioides* was more in this pairing method than in inoculation of antagonist earlier than pathogen. Highly significant F value ($p>0.0001$) for pairing methods showed that radial growth suppression of *F. verticillioides* by *T. pseudokoningii* strains significantly differed among pairing methods. Inoculation of antagonist earlier than pathogen resulted in the best growth inhibition of pathogen, this being shown by its higher significance over the other two pairing methods. This means that inoculation of *T. pseudokoningii* strains earlier than *F. verticillioides* caused the most effective radial growth suppression of pathogen. Inoculating *T. pseudokoningii* strains and *F. verticillioides* simultaneously also resulted in better radial growth suppression of *F. verticillioides* than inoculation of pathogen earlier than antagonist. This is shown by the significance of simultaneous inoculation of antagonist and pathogen compared to inoculation of pathogen earlier than antagonist.

For effective competitive exclusion of *F. verticillioides* from maize plant in the field, it might be better for any of *T. pseudokoningii* strains to be well established within maize plant ahead of the pathogen. It might also be better for them to occur simultaneously with *F. verticillioides* within maize plant than for pathogen to be established earlier. By this, they might be able to attack pathogen early before it does any substantial damage to the host plant as reported by Sharma and Sankaran (1988). The *T. pseudokoningii* strains would probably use a combination of biocontrol mechanism against *F. verticillioides* within maize plant. The *T. pseudokoningii* strains could be used to treat maize seeds or soil before planting to check incidence of endophytic *F. verticillioides* in maize plant. *T. harzianum* applied to seeds spread along plant roots and rhizosphere, subsequently reducing necrosis in aerial part of growing pepper plants (Ahmed et al. 2000). Any of these *T. pseudokoningii* strains especially strains 3 and 4 could produce such a systemic protection effect thereby checking stem and/or root rot or even ear rot of maize caused by *F. verticillioides*. The strains could be useful in biological control as pre-harvest agents to prevent disease during plant development. They might also be tried as post-harvest agents in suppressing production of fumonisins during seed storage. However, more work would have to be done before this could be established. Success of the 5 strains of *T. pseudokoningii*, which were isolates from maize plant parts and its rhizosphere, in growth inhibition of *F. verticillioides* supported the submission of Yates et al. (2000). In their experiments, *T. viride*, which successfully suppressed radial growth of *F. verticillioides*, was isolated from roots of maize plant. The fact that all strains of *T. pseudokoningii* were resident microorganisms of maize plant and its rhizosphere makes it easy for them to either occur before or simultaneously with endophytic *F. verticillioides*. Howell (2003) concluded that the best method for obtaining a potential biocontrol agent is to isolate the *Trichoderma* species from areas of plant and soil where it is expected to function in disease control. This coupled with their mechanisms for biocontrol (which include mycoparasitism and competition for space and nutrients) as well as their aggressiveness and high sporulation capacity and other desirable traits to make the five *T. pseudokoningii* strains promising as potential biocontrol agents for endophytic *F. verticillioides*. *T. pseudokoningii* strain 3 or

4, or even their hybrids (Howell 2003) look more promising than other strains in their antagonistic potential against endophytic *F. verticillioides* within maize plant. The study is part of continuous search for biocontrol agents that will remain tested in the field, for the competitive exclusion of *F. verticillioides* from maize plant.

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POLISH SUMMARY

HAMOWANIE WZROSTU *FUSARIUM VERTICILLIOIDES* (SACC.) NIRENBERG PRZEZ SZCZEPY *TRICHODERMA PSEUDOKONINGII* IZOLOWANE Z ROŚLIN KUKURYDZY ORAZ RIZOSFERY

W warunkach *in vitro* zbadano aktywność pięciu szczepów *Trichoderma pseudokoningii* w hamowaniu wzrostu fitopatogennego szczepu *Fusarium verticillioides* – sprawcy fuzaryjnego uwiądu kukurydzy. Każdy szczep antagonisty wysiewano w szalkach Petriego z grzybem chorobotwórczym i analizowano ich wzrost zgodnie z metodyką GLM(SAS).

Najsilniejsze hamowanie wzrostu *F. verticillioides* stwierdzono, gdy antagonista był inokulowany jako pierwszy. Szczep nr 3 *T. pseudokoningii* wykazał najsilniejsze działanie i może być przydatny w biologicznej ochronie kukurydzy przed fuzaryjnym uwiądem.