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Abilities of *Trichoderma* Species to Persist within Maize (*Zea mays*) Stem Long after Inoculation

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Research Article

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ABSTRACT

Different *Trichoderma* species were examined for their abilities to persist within the maize (*Zea mays*) stem at different points above and below inoculation points. Different *Trichoderma* species were isolated from different parts of the maize (*Z. mays*) plant and its rhizosphere. They were later sent to International Mycological Institute, England for identification. Maize seeds (DMR-LSRW) were planted in pots in the screenhouse. Four weeks after planting, each of the *Trichoderma* species was inoculated into the stems of the potted plants at the 2nd internodes using the toothpick method. Toothpicks dressed with sterile distilled water served as control. Cut sections of the inoculated stems were examined for presence or absence of the inoculated *Trichoderma* species at different points far from the inoculated point in the upper and lower internodes after 2, 3, 4, 5 and 6 weeks of inoculation. Ten *Trichoderma* species were identified; these include five strains of *T. pseudokoningii*, three strains of *T. harzianum, T. hamatum* and *T. longibrachiatum*. All the *Trichoderma* species were able to move within the stem tissues into the upper and lower internodes. All of them were re-isolated at distant points from inoculation point in the upper and lower internodes even after 6 weeks of inoculation. *T. pseudokoningii* strain 2 and *T. harzianum* strains 1 to 3 had the best endophytic movement into the upper and

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lower internodes. *T. hamatum* and *T. longibrachiatum* had the weakest movement into the upper and lower internodes. All the *Trichoderma* species could thus be said to possess the abilities to persist (endophytic capability) within the maize (*Z. mays*) stem. *T. pseudokoningii* and *T. harzianum* could also be said to be among the best species in the genus *Trichoderma* with good prospect of biocontrol potential.

Keywords: Trichoderma species; T. pseudokoningii; T. harzianum; lower internodes; upper internodes;

1. INTRODUCTION

Maize, because of its worldwide distribution and lower price relative to other cereals is known to have a wider range of uses than any other cereal (Bunting *et al*., 1978). *Trichoderma* species has been reported to be among the most commonly used biocontrol fungi against pathogens (Paavanen-Huhtala et al., 2000). The genus *Trichoderma* had actually produced tested fungal antagonists against several pathogens of many crops (Etebarian et al., 2000). Their successful biocontrol records was reported to be due to their ability to parasitize other fungi (Howell, 2003). However, the consistency of a good antagonist against a pathogen both in the laboratory and in the field is important and has been linked to the persistent and aggressive ability of such an antagonist. Biological control agents had often been reported to be less efficacious than the chemical fungicides under field conditions due to inability of such biocontrol agent to establish itself in the target host (Burgess and Keane, 1997; Mathre et al., 1994; Mao et al., 1997).

Amongst other characteristics, in selecting a microorganism as a biocontrol agent, the availability of a suitable antagonist capable of maintaining itself on the host plant is of prime importance (Sharma and Sankaran, 1988).

The objectives of the study therefore was to examine the abilities of the different *Trichoderma* species that successfully inhibited growth of *Fusarium verticillioides in vitro* (Sobowale et al., 2005) to persist in the maize (*Zea mays*) internodes at varying distances above and below inoculation point in the screenhouse.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of *Trichoderma* Species

Different *Trichoderma* species were isolated from different parts of maize plant and its rhizosphere. Isolation methods include 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) and they were sent to International Mycological Institute (IMI) England for identification purposes.

2.2 Planting and Inoculation of Maize (*Zea mays*) Stem in the Screenhouse

Maize seeds (DMR-LSRW) were planted in twenty pots (four seeds per pot later thinned down to two). Experimental design employed was a Complete Randomized Design (CRD). The soil that was used in the screenhouse was the normal untreated soil from the field. Each of the *Tricoderma* species was grown on toothpicks using the method described by Sobowale et al. (2007) Toothpicks dressed with sterile distilled water served as control. Four weeks after planting and watering on a regular basis, sterilized nail on a wooden handle was used to pierce the stems of the potted plants at the 2nd internodes and the toothpicks (dressed with each *Trichoderma* species) were inserted therein using a sterile forceps. Toothpicks dressed with sterile distilled water (control) were inserted into the stems of the other potted plants at the same position.

2.3 Data Collection

Two weeks after inoculation, maize stems from two pots were observed for any form of change whatsoever that might have been caused by the inoculated antagonist. Cut sections of the inoculated stems, of specific distances of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20cm above inoculation point (in the upper internodes), and 2, 4, 6, 8 and 10cm below inoculation point (in the lower internodes) were made using sterile knives and were brought back to the laboratory inside sterile brown paper bags to check the possibility of recovering each of the *Trichoderma* species at distances far from the inoculation points in the upper and lower internodes (Sharma and Sankaran, 1988). In the laboratory, the different cut sections of the stems, of specific distances from point of inoculation were surface sterilized by soaking in 10% sodium hypochloride (NaOCl) for about 5 minutes and then rinsed in five changes of sterile distilled water. They were later transferred into sterile filter papers (using sterile forceps) with which they were dabbed for about 90 seconds (i.e., until the cut sections were dried). Afterwards, they were picked unto sterile prepared APDA plates using sterile forceps and the plates were incubated at about 28^0C for two to seven days. The plates were later observed for the growth of each of the *Trichoderma* species from different cut sections of known distances from the point of inoculation. Same thing was done on the 3rd, 4th, 5th and 6th week after inoculation. Cut sections of stems that received treatment of sterile water (control) was also treated the same.

3. RESULTS

Ten *Trichoderma* species were identified; these are *T. pseudokoningii* strain 1, *T. harzianum* strain 1, *T. harzianum* strain 2, *T. hamatum, T. pseudokoningii* strain 2, *T. harzianum* strain 3, *T. pseudokoningii* strain 3, *T. pseudokoningii* strain 4, *T. longibrachiatum* and *T. pseudokoningii* strain 5. All the *Trichoderma* species were re-isolated from the stem tissues at varying distances from point of inoculation in the upper and lower internodes for the whole period of the six weeks. For the whole period of the six weeks, all the *Trichoderma* species continued to move, up into the upper internodes and down into the lower internodes covering varying distances from inoculation points (Figure 1). All the Trichoderma species were consistently re-isolated at points far from the inoculation points in the upper and lower internodes from the first week to the sixth week after inoculation. *T. pseudokoningii* strain 2 and the three strains of *T. harzianum* (Plates 1 and 2) had better endophytic abilities than the other *Trichoderma* species. They were recovered from as far as 20cm from inoculation point in the upper internodes and 10cm from inoculation point in the lower internodes. T. pseudokoningii strains 1 and 2 consistently had better endophytic ability in the upper

internodes than in the lower internodes (Figure 1). *T. pseudokoningii* strains 4 and 5 had close endophytic ability in both the upper and lower internodes (Plate 3). *T. hamatum* and *T. longibrachiatum* had the weakest persistence in the stem tissues, having been able to move not more than10cm above and below inoculation points (Figure 1).

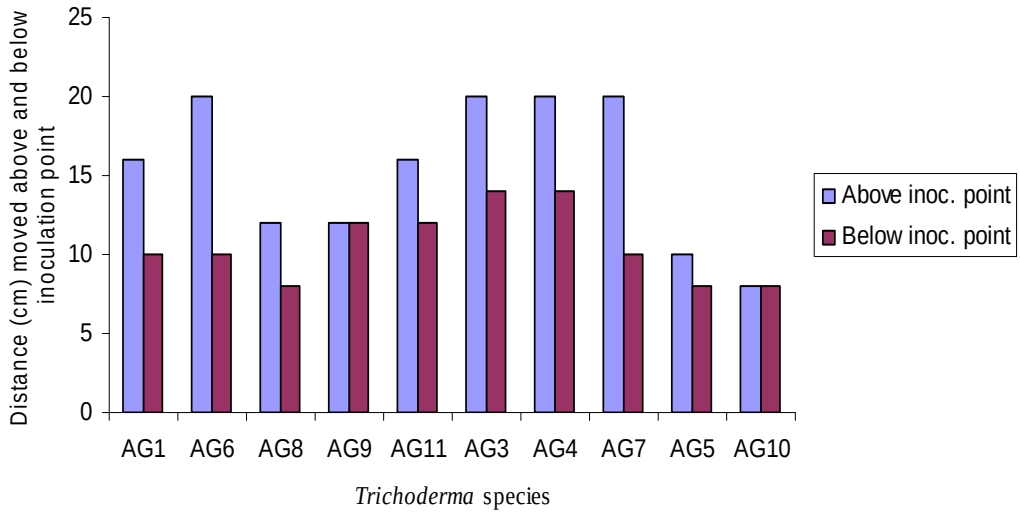
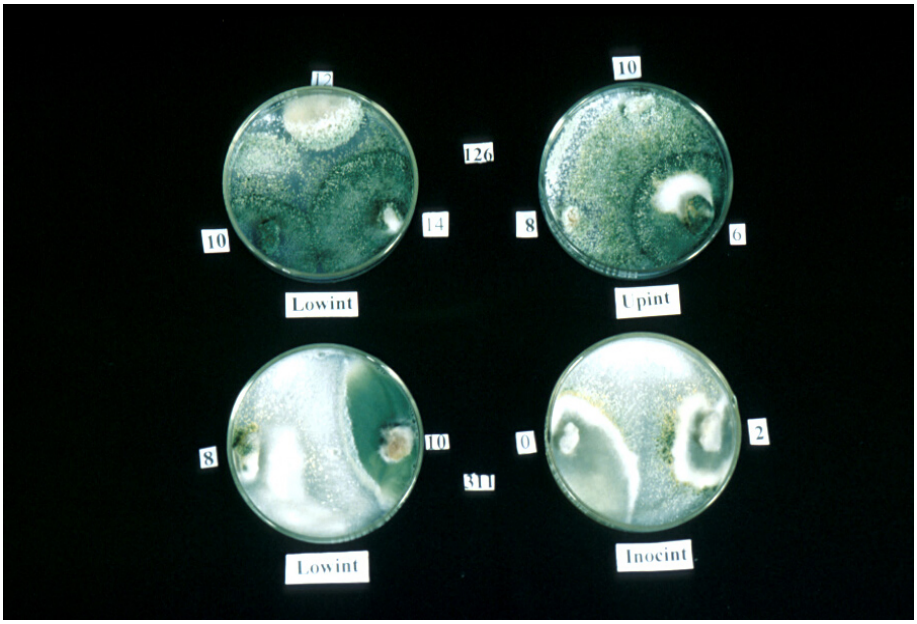


Fig. 1. Abilities of different *Trichoderma* species to persist at varying distances above and below inoculation point after 6 weeks of inoculation

AG1: T. pseudokoningii strain 1;
AG6: T. pseudokoningii strain 2
AG8: T. pseudokoningii strain 3
AG9: T. pseudokoningii strain 4
AG11: T. pseudokoningii strain 5
AG3: T. harzianum strain 1;
AG4: T. harzianum strain 2
AG7: T. harzianum strain 3
AG5: T. hamatum
AG10: T. longibrachiatum



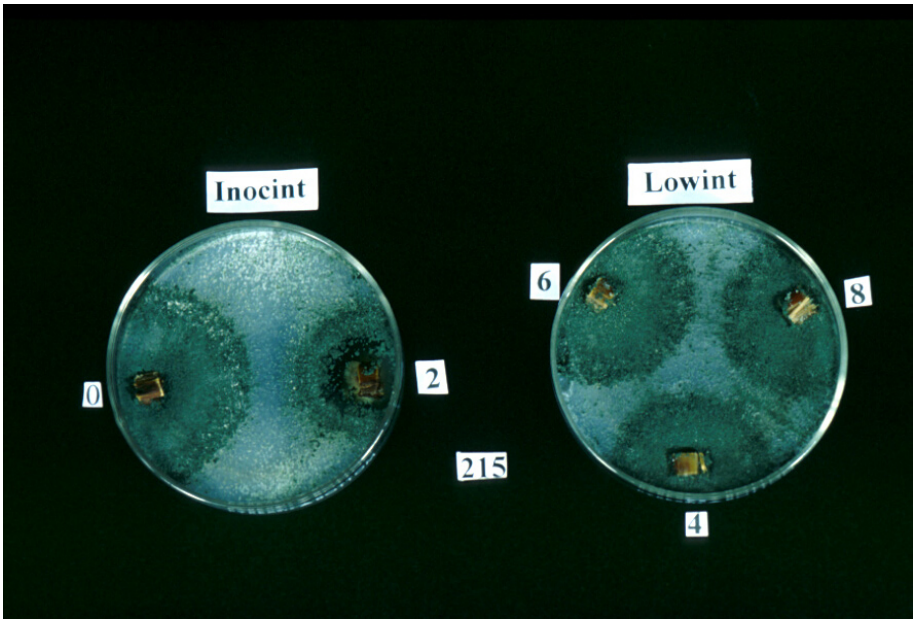


Plate 1. *T. harzianum* strains 1 (top) and 2 (bottom) recovered from varying distances from inoculation points in the inoculated internode (inocint) as well as upper (upint) and lower (lowint) internodes

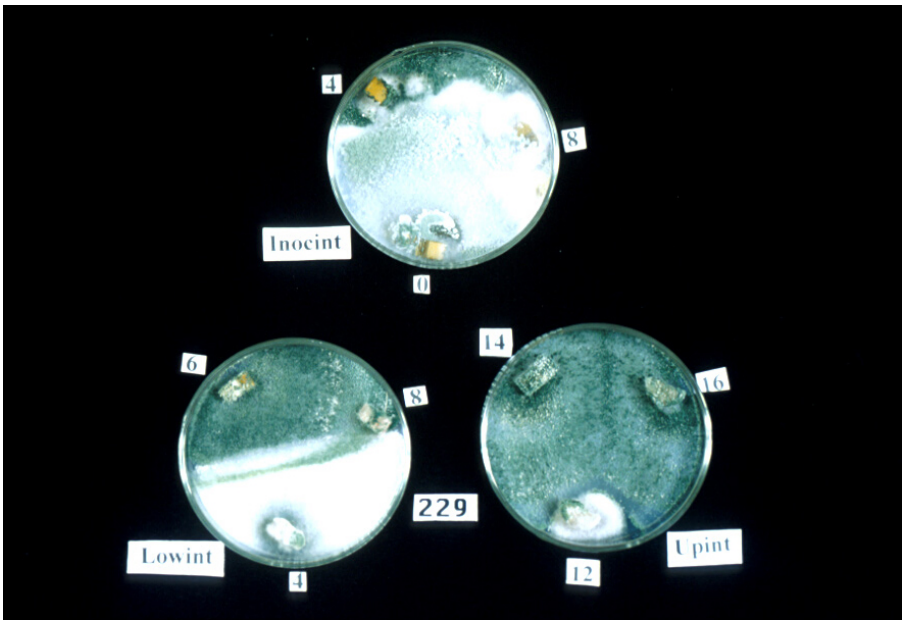
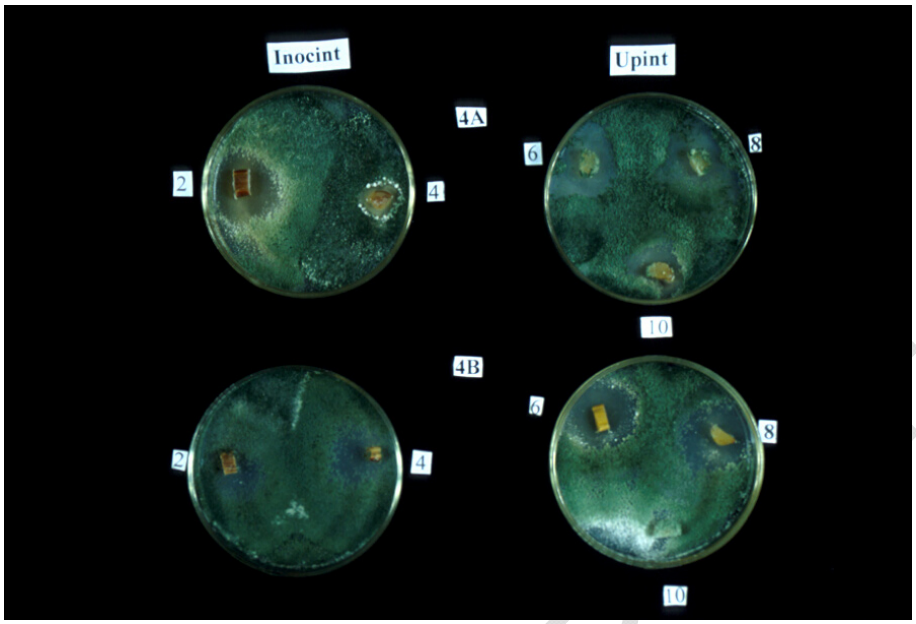


Plate 2. *T. harzianum* strain 3 recovered from varying distances from inoculation points in the inoculated internode (inocint) as well as upper (upint) and lower (lowint) internodes



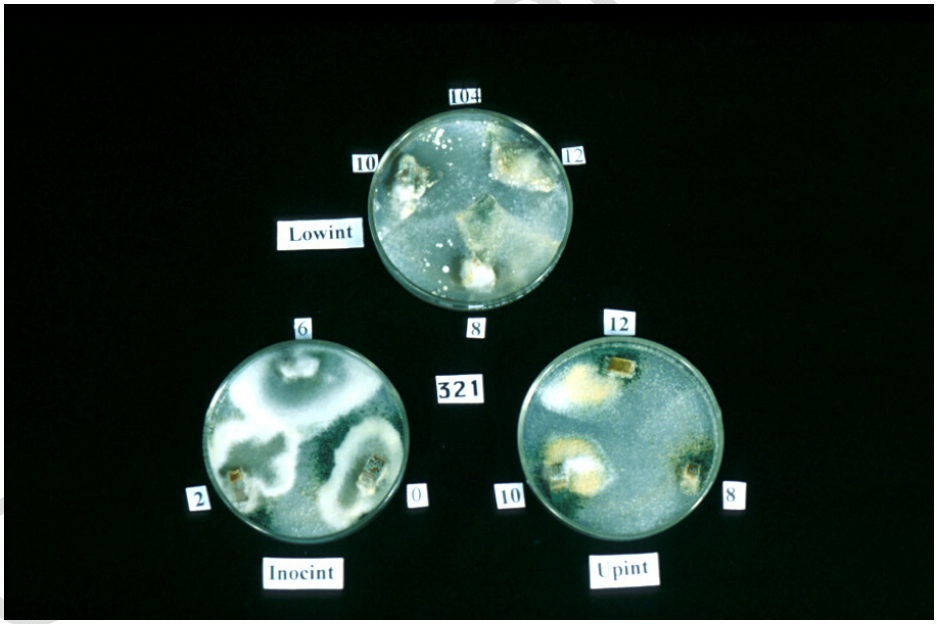


Plate 3. *T. pseudokoningii* strains 4 (top) and 5 (bottom) recovered from varying distances from inoculation points in the inoculated internode (inocint) as well as upper (upint) and lower (lowint) internodes

4. DISCUSSION

The persistence of the *Trichoderma* species within the stem tissues even at far distant points from point of inoculation agreed with the conclusions of Sharma and Sankaran (1988) that a good biocontrol agent should have a good degree of persistence and aggressiveness. The varying distances moved by different *Trichoderma* species within the stem tissues and their varying recoveries from different distant points from inoculation point showed the difference in the aggressive or endophytic capabilities of the different *Trichoderma* species. It could therefore be said that all the *Trichoderma* species possess the abilities to maintain themselves on the host plant. This, according to Sharma and Sankaran (1988) is of prime importance. The fact that all the *Trichoderma* species were re-isolated at distant points from inoculation point even after 6 weeks of inoculation also underscores their ability to persist within the stem tissues. This explains why *Trichoderma* species are generally used as biocontrol agents.

The genus *Trichoderma* has actually been reported by many researchers to be one of the most commonly used bioconrol fungi against several pathogens (Paavanen-Huhtala et al., 2000; Thrane et al., 2000). Their ability to parasitize other fungi, as reported by Howell (2003) may also be linked to their ability to persist and to move a great distance within a given host at any point in time. Their fast and high sporulation rate, which is another trait of a good antagonist (Campbell, 1988), may also account for their good endophytic or aggressive capability. The better performance (in terms of movement) of *T. pseudokoningii* strain 2 and the three strains of *T. harzianum* could mean that these *Trichoderma* species may be among the best *Trichoderma* species with endophytic capabilities within the maize stem. This much was also corroborated in the experiments of Sobowale et al. (2005) and Sobowale et al (2007). This ability of the strains of *T. pseudokoningii* and *T. harzianum* to move endophytically in the maize (*Z. mays*) stem to distances far from the inoculated point may have also informed their success against such a systemic pathogen as *F. verticillioides* in vivo (Sobowale et al., 2007). Munkvold and Carlton (1996), Lawrence et al. (1981), Kingsland and Wernham (1962) amongst others confirmed the systemic ability of *F. verticillioides*.

5. CONCLUSION

Any of the ten *Trichoderma* species could thus be concluded to exhibit promising endophytic characteristics in the tissues of maize (*Zea mays*) stem. Specifically, *T. pseudokoningii* and *T. harzianum* could also be concluded to be among the best in the genus *Trichoderma* with a bright prospect of biocontrol potential against pathogens such as *F. verticillioides* and indeed other pathogens.

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