Archives Of Phytopathology And Plant Protection
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454295

Seed biopriming with novel strain of **Trichoderma harzianum** for the control of toxigenic **Fusarium verticillioides** and fumonisins in maize

S. Chandra Nayaka [a]; S. R. Niranjana [a]; A.C. Uday Shankar [a]; S. Niranjan Raj [a]; M. S. Reddy [b]; H. S. Prakash [a]; C. N. Mortensen [c]
[a] Asian Seed Health Centre, Department of Studies in Applied Botany, Seed Pathology and Biotechnology, University of Mysore, Manasagangotri, Mysore, Karnataka, India
[b] Department of Entomology and Plant Pathology, Auburn University, USA
[c] Danish Seed Health Centre for Developing Countries, Department of Plant Biology, Faculty of Life Sciences, Denmark

First Published on: 11 February 2008

To cite this Article: Nayaka, S. Chandra, Niranjana, S. R., Shankar, A.C. Uday, Raj, S. Niranjan, Reddy, M. S., Prakash, H. S. and Mortensen, C. N. (2008) 'Seed biopriming with novel strain of **Trichoderma harzianum** for the control of toxigenic **Fusarium verticillioides** and fumonisins in maize', Archives Of Phytopathology And Plant Protection,

To link to this article: DOI: 10.1080/03235400701803879
URL: http://dx.doi.org/10.1080/03235400701803879

Archives of Phytopathology and Plant Protection
2008; 1–19, i*First* article

Seed biopriming with novel strain of *Trichoderma harzianum* for the control of toxigenic *Fusarium verticillioides* and fumonisins in maize

S. Chandra Nayaka[a], S.R. Niranjana[a]*, A.C. Uday Shankar[a], S. Niranjan Raj[a], M.S. Reddy[b], H.S. Prakash[a] and C.N. Mortensen[c]

[a]*Asian Seed Health Centre, Department of Studies in Applied Botany, Seed Pathology and Biotechnology, University of Mysore, Manasagangotri, Mysore, Karnataka, India;* [b]*Department of Entomology and Plant Pathology, Auburn University, USA and* [c]*Danish Seed Health Centre for Developing Countries, Department of Plant Biology, Faculty of Life Sciences, Copenhagen University, Denmark*

(*Received 9 October 2007*)

Fusarium verticillioides is one of the most important fungal pathogens in maize causing both pre- and post-harvest losses and also capable of producing Fumonisins. In the present study attempts have been made for screening potential *T. harzianum* from native rhizosphere and to study its effect on Fusarium ear rot disease, fumonisin accumulation in different maize cultivars grown in India. Eight isolates of *T. harzianum* were isolated and *T. harzianum* isolate Th-8 exhibited better antifungal activity than carbendizim. Th-8 was formulated in different solid substrates like wheat bran, paddy husk, talcum powder and cornstarch. Maize seeds of kanchan (moderately resistant), pioneer (resistant) and sweet corn (susceptible) were selected for laboratory and field studies and these seeds were treated with a conidial suspension of *T. harzianum* at the rate of 1×10^8 spore/ml and formulation at the rate of 10 g/kg. Treated seeds were subjected to evaluate *F. verticillioides* incidence, seed germination, seedling vigour and field emergence, yield, thousand seed weight and fumonisin production. It was found that the pure culture of *T. harzianum* was more effective in reducing the *F. verticillioides* and fumonisin incidence followed by Talc formulation than the carbendizim treated and untreated control. Formulations of *T. harzianum* were effective at reducing the *F. verticillioides* and Fumonisin infection and also increasing the seed germination, vigour index, field emergence, yield, and thousand seed weight in comparison with the control.

Keywords: *Trichoderma harzianum*; *Fusarium verticillioides*; fumonisins; biocontrol

Introduction

During the growing season, maize crop is exposed to a wide array of environmental elements such as insects, diseases, chemicals, and management practices, which affect its growth and development. Before an evaluation of the problem can occur and control measures be implemented, the problem must first be positively identified.

Fusarium verticillioides is one of the most important fungal pathogen in maize. It causes both pre-and post-harvest losses. During pre-harvest, *Fusarium* rots are responsible for the loss of 8.6% of total production globally. Infected seeds usually germinate

*Corresponding author. Email: srn@appbot.uni-mysore.ac.in

ISSN 0323-5408 print/ISSN 1477-2906 online

DOI: 10.1080/03235400701803879
http://www.informaworld.com

normally and produce healthy seedlings, although *F. verticillioides* can cause damping-off of seedlings under certain conditions. In developing countries, the uncertified seed infection may be 100% as revealed by laboratory testing. In storage conditions, infected seeds are capable of producing several mycotoxins such as fumonisin, moniliformin, zearoleanone and trichothecene which will reduce the grain loss up to 20%. Unlike the other mycotoxins associated with corn, high Fumonisin concentrations have also been found in seemingly healthy corn kernels. Examination of such corn reveals that *Fusarium verticillioides* is present in these kernels, even though there are no visible symptoms or signs of disease. Fumonisins were discovered in 1988 and are produced by *F. verticillioides, F. proliferatum* and several uncommon *Fusaria* (Gelderblom et al. 1988; Nelson et al. 1993). Fumonisins have been found to be very common contaminants of corn-based food and feed in USA, China, Europe and South America (Miller 1999). They are toxic and known to cause encephalomalacia in horses (Prelusky et al. 1994). Exposure to *F. verticillioides* contaminated maize has been linked to elevated rates of esophageal cancer (Thiel et al. 1992).

Biological disease control is a promising strategy for managing seed-borne, soil-borne and/or foliar diseases in a wide range of crops. Effective, biological disease control depends not only on suitable biocontrol organisms but also on methods and strategies for introducing and maintaining population levels and their activities. Regardless of the activity of the biocontrol agents, the methods used to produce, formulate and deliver these organisms may profoundly influence their efficacy under field conditions.

One of the popular methods of introducing biological control agents is seed treatment. Applying microorganisms to seed is an attractive proposition because of the combination of specific effect and limited environmental impact. In the familiar adage, seed treatment has the potential to deliver agents ''in the right amount, at the right place, and at the right time'' (McQuilken et al. 1998).

With increasing public awareness of the potential environmental and health hazards of both agrochemicals and fertilizers and the advances in biotechnology to improve the performance of microbial products, application of microorganisms to seeds is likely to increase in the future. In view of this, we have attempted the use of *Trichoderma harzianum* as seed treatment for control of maize ear rot and management of fumonisin accumulation in maize seed samples.

Materials and methods

Isolation of Trichoderma harzianum *isolates*

A total of 15 soil samples were collected from different maize fields of Karnataka State, India during 2003 for the isolation of *T. harzianum*. These maize fields were established by local farmers under normal agronomic conditions. Soil samples were collected from the root surface of growing maize seedlings. Replicate samples from the field were collected and later combined. Samples were brought to the laboratory for isolation of microbial agents. The soil samples were air-dried at room temperature ($25 \pm 2^{\circ}C$) for 2–3 days then mixed thoroughly and sieved using 250 μ mesh-size.

About 10 g of each soil sample was suspended in 95 ml of sterile water, stirred well and kept for 20 min. From this stock suspension, 1 ml was taken and serially diluted with sterile distilled water up to 10^{-2} and 10^{-9} dilutions. From the required dilutions, 400 μl of each of the dilutions was taken and spread onto 90 mm glass petriplates containing THS medium which is highly selective for *T. harzianum* (Williams et al. 2003) and the plates were incubated at $25 \pm 1^{\circ}C$ under alternate cycles of 12/12 hours of Near Ultra Violet (NUV) for seven days.

The plates were observed regularly and the colonies of *T. harzianum* were identified by its morphological characters. They were further observed under a light microscope for confirmation based on the filamentous nature and the width of the hyphae. Individual colonies that appeared on THSM were picked up and subcultured on THSM slants/plates for working stock. Glycerol (30%) vials and freeze-dried ampoules were also prepared with the isolated *T. harzianum* and stored at -20°C.

A total of eight strains of *T. harzianum* were isolated from the soil samples and designated as ABTh1 to ABTh8.

Trichoderma harzianum

The cultures of *T. harzianum* were prepared from eight-day-old cultures and suspensions were adjusted to 1×10^8 spore/ml by using a haemocytometer.

Fusarium verticillioides

The toxigenic strain of *F. verticillioides* (AbFv-12) culture (obtained from the microbial stock culture collection of the Asian Seed Health Centre at the Department of Applied Botany and Biotechnology, University of Mysore, India) was prepared from eight-day-old cultures and suspensions were adjusted to 1×10^8 conidia/ml by using a haemocytometer.

In vitro *antagonistic activity of* T. harzianum *against* F. verticillioides

Micro-well dilution assay (MIC). The 96-well plates were prepared by dispensing into each well 95 µl of PDB culture media and 5 µl of the *F. verticillioides* inoculum. One hundred µl of *T. harzinaum* initially prepared at a concentration of 10^8 cfu/ml was added to the first wells. Then, 100 µl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 µl of PD broth without any organisms and 5 µl of the inoculum on each strip was used as negative control. The final volume in each well was 200 µl. Carbendizim at a concentration of 100 µl was prepared in PD broth and used as positive control; the plate was covered with a sterile plate sealer. The contents of each well were mixed on a plate shaker at 300 rpm for 1 minute and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by absorbance at 610 nm using the Elx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, Vermont, USA) and confirmed by plating 5 µl samples from clear wells on potato dextrose agar medium. The experiment was repeated twice and the MIC was defined as the lowest concentration of the *T. harzianum* required to inhibit the growth of *F. verticillioides*.

Dual culture method. In this assay 10 mm PDA culture disc of the pathogen was cut individually from seven-day-old culture. This was placed on one side of the previously plated sterilized PDA medium approximately 1.5 cm away from the edge of the plate. Simultaneously, culture disc of the *T. harzinaum* was placed on to the opposite side. Five replications were maintained. The plates were incubated at room temperature ($24 \pm 2^{\circ}$C) for seven days. The diameter of the mycelial growth of the pathogen was measured and the results were expressed in terms of percentage inhibition of mycelium over control.

Biocontrol efficacy of T. harzianum *against* F. verticillioides

Production of *T. harzianum* inoculum. *T. harzianum* isolate Th8 that exhibited the highest virulence against *F. verticillioides* under laboratory conditions was selected for inoculum production.

The inoculum of *T. harzianum* was prepared at the rate of 1×10^8 as detailed earlier.

Mass production of T. harzianum *(Th8) in liquid broth*. Inoculum of *T. harzianum* was produced in potato dextrose broth (pH 6.5). Conidial suspension (100 µl) of *T. harzianum* was inoculated into sterile 1000 ml Roux bottles containing 125 ml of PDB. The bottles were placed horizontally and incubated $25 \pm 2^{\circ}C$ under 12/12 h cycles of light and darkness. After 14 days of incubation under static conditions, the mycelial mat was harvested by decanting the spent broth. The mycelial mats were blot dried and the spores per gram of mycelial mat were determined by making a spore suspension in 0.02% tween 20. The spore load was adjusted to 1×10^8 by using a haemocytometer.

Mass production of T. harzianum *(Th8) on different solid substrates*

Wheat bran and paddy husk were selected based on their local availability, high protein and starch content and suitability for mass production as solid substrates. These substrates were collected from local food processing industries in and around Mysore city of Karnataka State.

Twenty grams of each substrate was placed in a 250 ml flasks containing distilled water. The contents were mixed thoroughly with the help of a glass rod until all the substrate particles were evenly moistened and no lumps were present. The flasks were autoclaved at 121°C and 15 lb pressure for 30 min. The flasks were inoculated with 1 ml of conidial suspension of *T. harzianum* containing 1×10^8 spores/ml. The contents were shaken well to evenly disperse the inoculum. The inoculated flasks were incubated at $25 \pm 2^{\circ}C$ under 12/12 h cycles of light and darkness for 14 days. During the incubation, the contents of the flasks were shaken once every day to prevent aggregation. The initial weight of the flasks before and after incubation was recorded; the fungus was harvested after 14 days of incubation when the substrate was completely overgrown with light green mycelium. At the time of harvest, 1 gram of mycelium was suspended in 9 ml of sterile distilled water containing 0.02% tween-20 to assess the concentration of the conidia using haemocytometer.

Formulation of T. harzianum *(Th8) for biocontrol efficacy*

Two different carrier materials, cornstarch and Talcum powder, were used for preparing formulations of *T. harzianum*.

For this, the mycelial mat (100 g) was transferred into a sterile beaker and mashed thoroughly to obtain a fine paste. The mycelial paste was used to prepare different formulations.

Cornstarch formulations were prepared by mixing gelatinized cornstarch and mycelial paste in a 1:1 ratio (w/w) (Perira and Roberts 1991). Gelatinized cornstarch was prepared by cooking cornstarch in distilled water. The cooked starch was autoclaved for 30 min at 121°C and mixed with ethanol to precipitate starch in the gelatinized form. The gelatinized starch was spread in steel trays and allowed to completely dry in a fume hood. The gelatinized cornstarch was blended for 2 min in a blender to obtain a fine powder.

The gelatinized cornstarch absorbed the culture medium and entrapped the fungal mycelia in a rubbery material. The formulation thus prepared was spread out on filter paper towels placed in a laminar air hood and allowed to dry for 4–5 hours, broken into small pieces and mixed with ungelatinized sterile cornstarch in the ratio 1:2 (formulation: cornstarch). The formulation was mixed in a blender, spread on a sterile filter paper towel in a fume hood and allowed to dry overnight. After drying the formulation was again broken into fine powder in a blender packed in polythene bags and stored at 4°C for further analysis. Samples of 1 g each were drawn after 30, 60, 90, 120 days and 12 months of storage. The samples were suspended in 9 ml of sterile distilled water containing 0.02% tween 20. Serial dilutions of the suspension were placed on PDA plates, incubated at 25 $\pm$ 2°C for 5 days and the number of colony forming units per gram of mycelium incorporated was determined.

Talcum formulation. Talcum powder formulation was prepared by mixing mycelial paste and sterile talcum material in the ratio 1:10 (w/w) (Rabindran and Vidyasekaran 1996). Prior to mixing of mycelial paste with talcum powder, 20 g of carboxymethylcellulose (adhesive) was added to 1 kg of both carriers and mixed well. The carriers were autoclaved at 121°C for 30 min on two consecutive trays. Then the mycelial paste was mixed thoroughly with the carriers under sterile conditions in a laminar hood. Formulations thus prepared were spread on sterile filter paper towel in a laminar hood and large clumps were broken. The formulations were allowed to dry for 12–16 h. Completely dry formulations were crushed in a blender for 1 min, packed in airtight polythene bags and stored at 4°C for further study. Samples of 1 g each were drawn after 30, 60, 90, 120 days and 12 months of storage. The samples were suspended in 9 ml of sterile distilled water containing 0.02% tween 20. Serial dilutions of the suspension were placed on PDA plates, incubated at 25 $\pm$ 2°C for 5 days and the number of spores/gram of mycelium incorporated was determined.

In both the formulations a conidial with a concentration of 1×10^8 conidia/ml was prepared in a aqueous solution of 0.02% tween 20 for laboratory and field evaluations.

Source of maize seeds

Maize cultivars of kanchan (moderately resistant), pioneer (resistant) and sweet corn (susceptible to *Fusarium*) were selected for laboratory and field studies. These samples were from All India Co-ordinated Maize Research Centre, Nagenahalli, Srirangapatna of Mandya District of Karnataka State.

Seed treatment

Maize seeds were treated with conidial suspension of *T. harzianum* at the rate of 1×10^8 spore/ml by mixing 400 seeds with 5 ml of conidial suspension. A formulation of *T. harzianum* (1×10^8 cfu/g) in the form of slurry was used to treat different cultivars of maize seeds at the rate of 10 g/kg of seeds. Carbendazim (methyl-2-benzimidazole carbamate), a standard fungicide recommended for maize seed protection in India, was treated at the rate of 2 g/kg of seeds for comparison. An untreated control was also maintained. After 24 h of treatment the seeds were air-dried and then were subjected to evaluate *F. verticillioides* incidence, seed germination, seedling vigour and field emergence, yield, thousand seed weight and fumonisin production.

Screening for incidence of F. verticillioides *in maize seeds*

Four hundred seeds of sweet corn, pioneer, kanchan were screened to record the percentage incidence of *F. verticillioides* by the standard blotter method (ISTA 2003).

Seed germination and Vigour Index test

Maize seeds treated with or without *T. harzianum* isolates as described earlier were subjected to a germination test according to the paper towel method (ISTA 2003). The vigour index was calculated according to the formula used by Abdul-Baki and Anderson (1973).

Efficacy of T. harzianum *(Th8) against* F. verticillioides *under greenhouse conditions*

Seeds of maize cultivars were taken in conical flasks and treated with pure culture of *T. harzianum* and its formulations at the rate of 1×10^8 spores/g of seed and incubated overnight at room temperature. Also seeds treated with the systemic fungicide carbendizim at the rate of 2.1% active ingredient at 6 g/kg of seed were also included as positive control. Seeds without *T. harzianum* served as untreated controls. The above seeds were sown in four plastic pots containing a mixture of soil and sand at a 2:1 ratio (v/v). There were five replications per treatment, and five pots per replication. They were arranged in a randomised complete block design. The emerging seedlings were challenge inoculated by whorl inoculation method with the spore suspension of *F. verticillioides* at a concentration of 1×10^{14} spores/ml prepared as described earlier. In the whorl inoculation method, droplets of *F. verticillioides* spores are dropped onto the leaf whorl formed by the emerging seedlings and allowed to flow down to the base. Pots were maintained under greenhouse conditions (90–95% RH, 22–25°C temperature) and observed for disease development. The seedlings were observed and rated for disease severity when they showed any one of the typical Fusarial infection like browning of leaves; necrotic lesions, damping-off and wrinkled leaves. After 15 days of sowing an average symptom expression was determined for each set of samples maintained individually.

Efficacy of T. harzianum *(Th8) as seed treatment under field conditions*

Field trials to test the efficacy of seed treatment with pure culture of *T. harzianum* and its formulation were conducted at Nagenahalli research station (All India Coordinated Maize Improvement Programme, Govt. India) at Srirangapatna (Tq), Mandya dist., Karnataka, The field had received no inoculum of any pathogens tested for three years. Field trials were conducted in three consecutive years during the crop seasons of 2003–2004, 2004–2005 and 2005–2006. For the entire field studies cultivars such as sweet corn, pioneer and kanchan, which are considered as susceptible to *F. verticillioides*, resistant and moderately resistant, respectively, were used.

In another set of experiments, pure culture and formulation of the *T. harzianum* were also applied as spray treatment at different stages of seed development such as boot leaf stage, anthesis stage, milky stage and physiologically mature stage at the rate of 19×10^8 spores/g. 1 g of powder formulation was dissolved in 100 ml of distilled water and each ear head was sprayed completely from the tip of the ear head at all the stages (Umesha et al. 1998), chemical fungicide carbendizim (0.2%) was also sprayed and distilled water plants were also maintained and considered as control. For all the

treatments 20 ear heads were selected from respective lines in four replicates. After complete physiological maturity, maize ear heads were harvested and dried in sunlight to a safe moisture level, threshed and packed in polythene bags.

Ear rot disease incidence

The trials were laid out in a randomised replicated block design and there were five replications in each treatment. Each replicated row was manually seeded with 100–150 seeds per row. These were arranged as a randomised complete block design. Normal agronomic practices were followed to raise the crop. Spacing was done after 21 days to maintain a uniform number of plants per row and uniform distance between the plants. The crop was irrigated once every 15 days. The plants were observed for ear rot/kernel rot disease development and rated for disease when they showed any one of the typical ear rot/kernel rots like a powdery or cottony-pink mold growth developing on the infected kernels. The data were consolidated at 60 days after sowing. Experiments were conducted twice. The disease was rated on a 0–7 scale following the method of Reid et al. (1996).

Ear rot and kernel rot was determined by counting the total number of cobs and the total number of infected cobs per plot. From these data, percentage of disease incidence and disease suppression were calculated. Data on percentages of incidence of ear rot and kernel rot were analysed by analysis of variance, and the least significant difference test (Gomez and Gomez 1984) was used for evaluating the significance of differences between the control and the treatments.

Effect of T. harzianum *(Th8) on growth promotion, yield and 1000 seed weight of maize in field conditions*

The field experiment was conducted to determine the effect of *T. harzianum* suspensions and powdered formulations on the growth of maize. Treatments were the same as described above. *T. harzianum* treated seeds were hand-sown and there were five replications per treatment. Each replication was a single row of 5 m length, hand seeded with 100–150 seeds per row. The field was maintained according to the maize growing conditions (red loamy soil, irrigated once every 15 days and spacing done after 21 days). No artificial chemicals or fertilizers were used. At 30 days after seeding, plant height was recorded (Niranjan Raj et al. 2003).

Maize yield. After complete maturity the maize cobs were harvested. The cobs that were harvested were dried and threshed separately. The grains thus obtained was weighed separately and the rate of infection control was calculated by calculating the accurate gain in the maize grain yield in the sprayed plots with different treatments compared to the unsprayed control plots.

1000 seed weight. Collected samples were also subjected to 1000 seed weight. From the working samples, eight replicates, each of 100 seeds, were randomly taken and counted with a seed counter. Each replicate was weighed in grains to the same number of decimal places. The variance, standard deviation and coefficient of variation were calculated as follows.

$$\text{Variance} = \frac{n(\varepsilon x^2) - (\varepsilon x^2)}{n(n-1)}$$

where x = weight of each replicate in grams, n = number of replicates, ε = sum of standard deviation (s) = root variance

$$\text{Coefficient of variation} = S \times \frac{100}{\text{X}}$$

The experiment was repeated for three consecutive years.

Screening of maize cultivars for resistance to Fusarium ear rot and kernel rot, accumulation of defense enzymes and fumonisin production

Fumonisins production. Seeds of different maize cultivars consisting of two resistant (pioneer and kanchan), and one susceptible i.e. sweet corn, seed samples were challenge inoculated with toxigenic *F. verticillioides*. The seed samples treated with *T. harzianum* pure cultures and formulations as described earlier and untreated control and carbendizim treated seeds were used to assess total fumonisins level by using commercially available quantitative ELISA assay kits (Neogen Corp., Lansing, MI, USA) were used for measuring the presence of total fumonisins according to the manufacturer's instructions.

Results

Isolation and identification of Trichoderma harzianum *isolates*

Among the 15 soil samples collected from different maize growing regions of Karnataka, *T. harzianum* were found only in eight samples.

In vitro antagonistic activity of T. harzianum *against* F. verticillioides

Micro well dilution assay

The antifungal activity of the isolates of *T. harzianum*, isolate Th-8 and Th-2 showed maximum inhibition when compared to carbendizim. The MIC values of Th-8 and Th-2 were 93 and 88, respectively (Figure 1a).

Antagonistic activity by dual culture method

The result of dual culture methods is shown in Figure 1b. The antagonists and *F. verticillioides* grew towards each other during the period of incubation. A zone of inhibition was observed between *F. verticillioides* and antagonists. Among all the isolates, Th-8 showed the maximum inhibition zone of 90 mm against *F. verticillioides* and restricted the growth of *F. verticillioides* upon contact inhibition.

The antifungal capability of *T. harzianum* isolate Th-8 exhibited better antifungal activity than carbendizim which is an extensively used fungicide for managing Fusarium diseases in India.

Effect of T. harzianum *(Th8) on incidence of* F. verticillioides *in three different cultivars of maize*

The effect of pure culture and different formulations of *T. harzianum* on percentage incidence of *F. verticilliodes* in treated seeds over control are presented in Table 1. Pure culture of *T. harzianum* at the rate of 1×10^8 spores/ml reduced the incidence by 79% in kanchan, 80.12% in pioneer and 68.7% in sweet corn. Different powder formulations of *T. harzianum*, viz., talcum powder formulation (63, 67 and 61%) cornstarch formulation

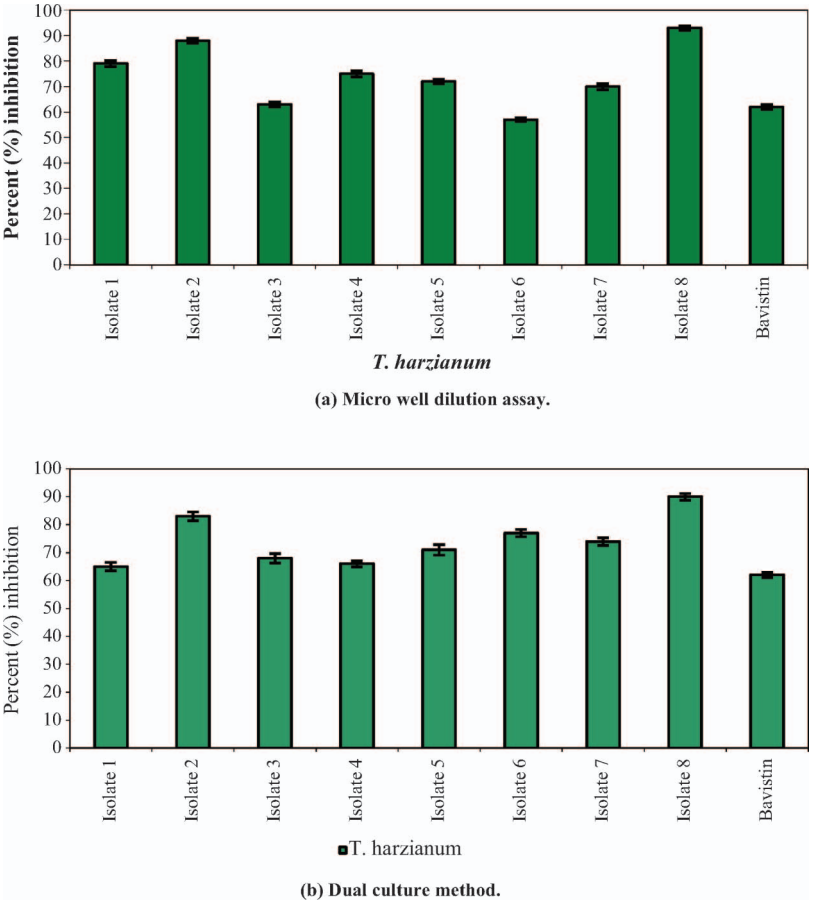

Figure 1. Antifungal activity of *T. harzianum* against *F. verticillioides* by different methods.

Table 1. Effect of various formulations of *T. harzianum* (Th8) on the incidence of *F. verticillioides* in different cultivars of maize under laboratory conditions.

Treatment *T. harzianum* (1×10^8)	Maize cultivars (% incidence)		
	Kanchan	Pioneer	Sweet corn
Pure culture	6.12^{a}	3.73^{a}	14.42^{a}
Talc formulation 10 g/Kg	10.84^{b}	5.42^{b}	16.47^{b}
Corn starch formulation 10 g/Kg	11.81^{c}	6.14^{c}	20.46^{c}
Paddy husk formulation 10 g/Kg	16.24^{d}	8.81^{d}	21.76^{de}
Wheat bran formulation 10 g/Kg	19.31^{e}	9.17^{e}	23.67^{f}
Carbendizim	12.01^{f}	7.37^{e}	17.75^{e}
Control	29.91^{g}	18.77^{e}	46.18^{g}

Values are means from three repeated experiments with four replications and 100 seeds per replication in each experiment. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

(60.7, 60.5 and 55.6%), paddy husk formulation (45, 53 and 58%) and wheat bran formulation (45, 53 and 58%), at the rate of 10 g/kg seeds reduced the incidence of *F. verticillioides* in kanchan, pioneer and sweet corn, respectively. Carbendizim also reduced the incidence of *F. verticillioides* by 56, 60 and 61% and was superior to the biocontrol powder formulations of paddy husk and wheat bran (Table 1).

Effect of seed treatment with T. harzianum *(Th8) on seed germination and vigour index*

T. harzianum strain (Th-8) as fresh suspensions or powdered formulations significantly enhanced germination and VI of maize seed under laboratory conditions compared to untreated controls. The highest enhancement rate of germination and vigour index was obtained with a pure culture of *T. harzianum* as seed treatment in both the application forms tested.

Pure culture of *T. harzianum* increased the seed germination and vigour index in all the cultivars, viz., kanchan 29.5, pioneer 18.2 and sweet corn 35.2% germination, 52.4, 36.0 and 66.4% of VI and talcum powder formulation gave an increase of 21, 12.1 and 23.5% of germination and 46, 33 and 61% of vigour index, followed by corn starch formulation 18.3, 7.3 and 17.6% of germination and 42, 31.4 and 60.1% of vigour index. Carbendizim treatment was superior compared to paddy husk and wheat bran and increased the germination by 18.3, 8.5 and 14.7%; the vigour index was increased by 40.7, 30.4 and 60% (Tables 2 and 3).

Carbendizim treatment also increased the seed germination (18.3, 8.5 and 14.7%) and VI (40.7, 50.5 and 60.0) followed by wheat bran, which increases the seed germination and vigour index by 12.6, 4.8 and 16.1 and 39.4, 26.2 and 58.9% (Tables 2 and 3).

Effect of seed treatment with T. harzianum *(Th8) on field emergence of maize cultivars*

An increase in field emergence was also observed in all cultivars of maize seeds treated with pure culture and formulations of *T. harzianum* when compared with carbendizim treated and untreated control during all the three years and is presented in Table 4.

Treatment of maize seeds with pure culture of *T. harzianum* at the rate of 1×10^8 cfu/ml concentration average increased the field emergence by 18.5, 21.5 and 18.7% in kanchan, pioneer and sweet corn, respectively. Whereas the talcum powder formulation

Table 2. Effect of *T. harzianum* (Th8) on seed germination in maize under laboratory conditions.

Treatment *T. harzianum* (1×10^8)	Maize cultivars (% germination)		
	Kanchan	Pioneer	Sweet corn
Pure culture	92^{a}	97^{a}	92^{a}
Talc formulation 10 g/Kg	86^{ab}	92^{b}	84^{b}
Corn starch formulation 10 g/Kg	84^{b}	88^{bc}	80^{c}
Paddy husk formulation 10 g/Kg	83^{bc}	86^{bc}	78^{cd}
Wheat bran formulation 10 g/Kg	80^{cd}	85^{cd}	74^{de}
Carbendizim	84^{g}	89^{hi}	78^{i}
Control	71^{gh}	82^{i}	68^{j}

Values are means from three repeated experiments with four replications and 100 seeds per replication in each experiment. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

Table 3. Effect of various formulations of *T. harzianum* (Th8) on vigour index of various cultivars of maize under laboratory conditions.

Treatments *T. harzianum* (1×10^8)	Maize cultivars I (vigour index)		
	Kanchan	Pioneer	Sweet corn
Pure culture	1948[a]	2158[a]	1864[a]
Talc formulation 10 g/kg	1876[b]	2110[b]	1807[b]
Corn starch formulation 10 g/kg	1823[c]	2078[c]	1794[c]
Paddy husk formulation 10 g/kg	1768[d]	2041[d]	1783[d]
Wheat bran formulation 10 g/kg	1751[d]	2008[e]	1781[d]
Carbendizim	1799[i]	2065[i]	1792[j]
Control	1278[i]	1586[j]	1120[k]

Values are means from three repeated experiments with four replications and 100 seeds per replication in each experiment. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

Table 4. Effect of various formulations of *T. harzianum* (Th8) on emergence of various cultivars of maize under field conditions.

Treatments *T. harzianum* (1×10^8)	Maize cultivars I (vigour index)		
	Kanchan	Pioneer	Sweet corn
Pure culture	80[a]	90[a]	79[a]
Talc formulation 10 g/kg	80[ab]	78[ab]	73[b]
Corn starch formulation 10 g/kg	75[abc]	77[ab]	71[b]
Paddy husk formulation 10 g/kg	76[bcd]	75[bc]	69[bc]
Wheat bran formulation 10 g/kg	76[bcd]	75[cd]	69[bcd]
Carbendizim	80[a]	90[a]	79[a]
Control	80[ab]	78[ab]	73[b]

Values are means from three repeated experiments with four replications and 100 seeds per replication in each experiment. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

treated seeds increased the field emergence by 10, 17.0 and 10.9%, cornstarch formulation 5.7, 6.5 and 7.8%, paddy husk formulation 4.2, 1.3 and 6.2%. Wheat bran formulation increased the field emergence by 4.2, 5.0 and 4.6% in kanchan, pioneer and sweet corn, respectively. Carbendizim treatment also increased the field emergence by 5.7, 5.2 and 4.6% in kanchan, pioneer and sweet corn, respectively (Table 4).

Effect of seed treatment with T. harzianum *(Th8) on plant height of maize cultivars*

T. harzianum tested with a pure culture or formulation as seed treatment showed positive growth responses under field conditions compared to carbendizim and untreated controls. Specifically, both pure culture and formulation of *T. harzianum* as seed treatment + spray treatment increased the plant height to a maximum extent. Pure culture of *T. harzianum* increased the height by 15% in kanchan, by 9.3% in pioneer and by 17.5% in sweet corn and treatments significantly enhanced seedling height in all the cultivars tested compared to formulation, carbendizim and untreated control (Table 5).

Seed treatment + spray treatment of talcum powder formulation of *T. harzianum* increased the plant height of maize cultivars, viz., kanchan, pioneer and sweet corn by

12.5%, 5.8% and 13.5%, in the case of *T. harzianum* followed by cornstarch formulation of *Th* (7.5, 2.32 and 8.1%) paddy husk formulation of *Th* (6.25, 1.16 and 8.1%) and wheat bran formulation of *Th* (6.2, 2.3 and 6.7%). Carbendizim also increased the plant height by 8.75 in kanchan, 2.32 in pioneer and 8.1% in sweet corn and was superior to paddy husk and wheat bran formulation of *T. harzianum* (Table 5).

Effect of seed treatment with T. harzianum *(Th8) on incidence of ear rot disease*

Under experimental plot conditions, significant reduction of ear rot disease was observed in the test rows raised from seeds treated with both pure culture and formulations of the *T. harzianum* when compared with the fungicide treated and untreated control rows.

The results showed that there was significant reduction in the incidence of ear rot disease in all the cultivars with seed treatment followed by spray treatment of pure culture and powder formulation of *T. harzianum*. Seed treatment followed by spray treatment of pure culture of *T. harzianum* reduced the incidence of ear rot disease in all the cultivars, viz. kanchan (73%) pioneer (69%) and sweet corn (84%), followed by talcum powder formulation (69, 59.4 and 81.8%), corn starch formulation, (68, 54, 81%) paddy husk formulation (64, 55 and 81%) and wheat bran formulation (60, 51 and 79%) (Table 6).

The chemical fungicide carbendizim as seed treatment followed by spray treatment also reduced the disease incidence in all the cultivars in kanchan by 70%, pioneer 53% and in sweet corn by 77%, which was more effective than paddy husk and wheat bran formulations. In all the cases foliar spray with different formulations without seed treatment also controlled ear rot disease. When the formulations were sprayed on plants grown from treated seeds, the effectiveness was higher than when spraying was carried out without any previous seed treatment.

Effect of T. harzianum *(Th8) on yield and 1000 seed weight of maize*

The untreated control plots yielded an average of 2123 kg/ha. All the treatments increased maize yield when compared to the carbendizim treated and untreated control (Tables 7 and 8).

Table 5. Effect of *T. harzianum* (Th8) on plant height of maize.

Treatments *T. harzianum* (1×10^8)	Maize cultivars (height in cm)		
	Kanchan	Pioneer	Sweet corn
Pure culture	88.3^{a}	90.6^{a}	81.2^{a}
Talc formulation 10 g/kg	86.4^{b}	89.4^{b}	79.3^{b}
Corn starch formulation 10 g/kg	85.7^{b}	88.2^{b}	77.6^{b}
Paddy husk formulation 10 g/kg	85.0^{b}	87.1^{b}	76.5^{c}
Wheat bran formulation 10 g/kg	84.1^{c}	87.3^{c}	76.0^{d}
Carbendizim	85.9^{f}	88.0^{e}	76.9^{j}
Control	80.2^{g}	86.2^{f}	74.1^{j}

Values are means from three repeated experiments with four replications and 100 seeds per replication in each experiment. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

Table 6. Effect of *T. harzianum* (Th8) treatment on incidence of ear rot disease in three different cultivars of maize.

Treatments *T. harzianum* (1×10^8)	Maize cultivars (% incidence)		
	Kan	Pion	S. corn
Seed Treatment	6.12^{a}	5.84^{a}	6.03^{a}
Foliar spray	7.98^{ab}	6.32^{b}	6.41^{b}
ST + FS	4.99^{abc}	3.92^{b}	5.08^{b}
Talc formulation 10 g/kg			
Seed Treatment	6.83^{bcd}	6.11^{b}	6.25^{bc}
Foliar spray	8.08^{bcd}	6.46^{bc}	6.73^{bc}
ST + FS	5.89^{bcd}	5.27^{bc}	5.81^{bcd}
Corn starch formulation 10 g/kg			
Seed Treatment	7.32^{cde}	6.49^{bc}	6.47^{bcde}
Foliar spray	8.64^{cde}	6.96^{bc}	6.76^{bcde}
ST + FS	8.01^{def}	5.93^{bc}	5.98^{bcde}
Paddy husk formulation 10 g/kg			
Seed Treatment	8.09^{def}	6.91^{bc}	6.83^{bcde}
Foliar spray	8.96^{def}	7.14^{bc}	6.97^{bcde}
ST + FS	6.68^{def}	6.16^{c}	6.18^{bcde}
Wheat bran formulation 10 g/kg			
Seed Treatment	8.11^{def}	6.91^{c}	6.98^{cde}
Foliar spray	8.78^{def}	7.19^{c}	7.09^{cde}
ST + FS	7.01^{f}	6.41^{c}	6.53^{de}
Carbendizim	5.59^{f}	6.10^{c}	7.14^{e}
Control	19.4^{g}	13.2^{d}	32.0^{f}

Values are means from three repeated experiments with four replications and 100 seeds per replication in each experiment. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

Among the different treatments seed + spray treatment of pure culture of *T. harzianum* increased the yield by 18.4%, 13.3% and 30.4% in kanchan, pioneer and sweet corn, respectively.

T. harzianum powder formulation, seed treatment followed by spray treatment of talcum formulation, increased the yield in all the cultivars viz., kanchan (14.4%) pioneer (10%) and sweet corn (26%), cornstarch formulation (11.8, 7.7 and 21.7%), paddy husk formulation (9.2, 5.5 and 13%) and wheat bran formulation (7.8, 4.1 and 8.6%). The chemical fungicide carbendizim also increased the maize yield in all the cultivars tested by 10.5, 6.6 and 15.2% respectively, which was more effective than paddy husk and wheat bran formulation.

Effect of different treatments on 1000 seed weight, which is the indicator of yield of grains, is given in (Table 8). There was a significant increase in 1000 seed weight in all the cultivars with the seed treatment followed by spray treatment. *T. harzianum* increased 1000 seed weight by 20, 17 and 29% in kanchan, pioneer and sweet corn, respectively. The chemical fungicide carbendizim increased the 1000 seed weight by 11, 5 and 17.1% in kanchan, pioneer and sweet corn, respectively.

Effect of T. harzianum *(Th8) seed treatment on Fumonisins level in maize samples*

The results showed that there was significant reduction in the incidence of total fumonisins level in all the cultivars with seed treatment followed by spray treatment of pure culture

Table 7. Effect of *T. harzianum* (Th8) treatments on yield in three different cultivars of maize.

Treatments *T. harzianum* (1×10^8)	Maize cultivars (% incidence)		
	Kan	Pion	S. corn
Seed Treatment	85.4[a]	95.0[a]	55.7[a]
Foliar spray	77.3[a]	89.9[b]	45.3[b]
ST + FS	90.8[b]	104.2[c]	59.2[b]
Talc formulation 10 g/kg			
Seed Treatment	78.5[b]	93.1[c]	51.8[b]
Foliar spray	74.9[c]	89.6[d]	50.0[b]
ST + FS	84.2[c]	99.3[d]	53.1[b]
Corn starch formulation 10 g/kg			
Seed Treatment	78.1[d]	91.1[e]	48.9[c]
Foliar spray	73.8[d]	89.2[e]	45.7[c]
ST + FS	82.1[e]	95.0[f]	52.0[d]
Paddy husk formulation 10 g/kg			
Seed Treatment	77.1[f]	89.3[f]	47.3[e]
Foliar spray	70.3[f]	86.9[g]	45.1[e]
ST + FS	80.1[f]	93.2[g]	49.2[e]
Wheat bran formulation 10 g/kg			
Seed Treatment	74.6[g]	85.1[h]	45.8[f]
Foliar spray	70.1[h]	83.0[i]	42.9[f]
ST + FS	78.9[i]	91.3[i]	46.7[g]
Carbendizim	79.2[j]	93.8[j]	49.2[h]
Control	73.0[k]	87.0[k]	45.0[i]

Values are means from three repeated experiments with four replications and 100 seeds per replication in each experiment. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

and powder formulation of *T. harzianum*. Seed treatment followed by spray treatment of pure culture of *T. harzianum* reduced the incidence of fumonisins level in all the cultivars viz., kanchan (66.6%) pioneer (56.4%) and sweet corn (85.8%), followed by talcum powder formulation (62, 50.6 and 73.4%), corn starch formulation (51.8, 37 and 75.5%), paddy husk formulation (48.3, 36.3 and 73.6%) and wheat bran formulation (47.7, 36.3 and 73.6%) (Table 9). The chemical fungicide carbendizim as seed treatment followed by spray treatment also reduced the disease incidence in all the cultivars in kanchan by 53%, in pioneer by 55% and in sweet corn by 79%, which was more effective than paddy husk and wheat bran formulations.

In both the treatment foliar spray with different formulations, without seed treatment also controlled fumonisins level in all the cultivars. When the formulations were sprayed on plants grown from treated seeds, the effectiveness was higher than when spraying was carried out without any previous seed treatment.

Discussion

In the present study 15 soil samples collected from different maize growing regions of Karnataka were screened for the isolation *T. harzianum*. Eight isolates of *T. harzianum* (Th1–Th8) were identified based on the colony morphology. These observations on colony characters showed no difference from those made earlier by Domsch et al. (1980), Martha (1992) and Vidyasekaran and Muthamilan (1995). Isolates were used to study the *in vitro*

Table 8. Effect of *T. harzianum* (Th8) on thousand seed weight in three different cultivars of maize.

Treatments *T. harzianum* (1×10^8)	Maize cultivars (% incidence)		
	Kan	Pion	S. corn
Seed Treatment	32.7^{a}	34.9^{a}	26.5^{a}
Foliar spray	30.2^{b}	33.0^{b}	25.3^{ab}
ST + FS	33.6^{bc}	36.7^{bc}	28.7^{bc}
Talc formulation 10 g/kg			
Seed Treatment	31.8^{bc}	33.7^{bc}	25.2^{cd}
Foliar spray	30.0^{cd}	32.9^{bc}	24.3^{cd}
ST + FS	32.5^{de}	35.5^{bc}	27.3^{d}
Corn starch formulation 10 g/kg			
Seed Treatment	30.9^{de}	33.2^{bcd}	25.1^{d}
Foliar spray	29.2^{fg}	32.8^{bcde}	23.9^{e}
ST + FS	31.1^{fg}	33.5^{bcde}	27.0^{e}
Paddy husk formulation 10 g/kg			
Seed Treatment	29.8^{g}	33.0^{bcde}	24.2^{e}
Foliar spray	28.3^{g}	32.8^{cde}	23.3^{e}
ST + FS	30.4^{g}	33.5^{cde}	25.9^{e}
Wheat bran formulation 10 g/kg			
Seed Treatment	28.6^{gh}	31.8^{de}	23.9^{f}
Foliar spray	28.4^{i}	32.5^{de}	22.7^{g}
ST + FS	29.0^{j}	33.2^{f}	25.5^{h}
Carbendizim	30.4^{k}	32.8^{g}	25.8^{i}
Control	27.9^{l}	32.5^{h}	22.4^{j}

Values are means from three repeated experiments with four replications and 100 seeds per replication in each experiment. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

antagonistic activity and its effect on seed germination in laboratory conditions. Among the eight isolates, isolate Th8 showed maximum *in vitro* antagonism against *F. verticillioides* and increased the maize seed germination and plant height to a greater extent compared to other isolates, hence this isolate was used for field studies.

A significant difference between antagonistic natures of microbial agents was noticed; *T. harzianum* exhibited mycoparasitism against *F. verticillioides* as it comes in contact with the pathogen and gets locked at the region of contact. The results obtained in the present study showed a different degree of antagonism of the same antagonistic agent against *F. verticillioides*; this agrees with the findings of Biswas et al. (1999) and Jagadeesh Kumar et al. (1995). A highly significant difference between the isolates of *T. harzianum* and various pathogen interactions was reported by Elad et al. (1980). In the present study, we could observe that *T. harzianum* inhibited spore formation of *F. verticillioides* when grown in dual culture. Similar findings were reported by Dubey and Patel (2001); isolates of *T. viride*, *T. harzianum* and *Gliocladium virens* inhibited the sclerotial formation of *Thanetophorus cucumeris* when grown in dual culture. Other workers (Roy 1977; Dubey 1998a, 1998b; Singh et al. 2000; Pratibanda et al. 2002) have reported the effectiveness of *T. viride* and *T. harzianum in vitro*.

Daily measurements of *F. verticillioides* colony diameter alone and in dual cultures with the isolate of *T. harzianum* showed antagonism to *F. verticillioides*. The isolate of the *Trichoderma* sp. suppressed growth of *F. verticillioides* colonies with time, increasing from

Table 9. Effect of *T. harzianum* (Th8) on Fumonisin level in three different cultivars of maize.

Treatments *T. harzianum* (1×10^8)	Maize cultivars (% incidence)		
	Kan	Pion	S. corn
Seed Treatment	92.1[a]	20.2[a]	178.34[a]
Foliar spray	96.8[b]	23.1[b]	185.43[a]
ST + FS	77.8[c]	15.10[c]	124.61[ab]
Talc formulation 10 g/kg			
Seed Treatment	98.7[d]	21.71[d]	209.10[b]
Foliar spray	102.1[e]	25.4[d]	217.13[b]
ST + FS	86.5[f]	17.13[e]	173.47[c]
Corn starch formulation 10 g/kg			
Seed Treatment	122.5[g]	23.21[f]	218.56[c]
Foliar spray	134.1[h]	26.50[g]	224.73[cd]
ST + FS	109.1[i]	21.84[h]	184.46[e]
Paddy husk formulation 10 g/kg			
Seed Treatment	129.7[j]	23.71[h]	238.47[e]
Foliar spray	145.8[k]	27.0[i]	263.54[e]
ST + FS	120.4[k]	22.6[i]	231.31[ef]
Wheat bran formulation 10 g/kg			
Seed Treatment	138.1[k]	25.25[i]	254.45[f]
Foliar spray	147.8[l]	27.9[i]	277.21[g]
ST + FS	121.7[l]	22.10[i]	231.48[h]
Carbendizim	109.2[l]	22.40[j]	186.65[i]
Control	234.0[l]	34.80[j]	878.45[j]

Values are means from three repeated experiments with four replications and 100 seeds per replication in each experiment. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

the 46% suppression observed on day 6 to a maximum of 91% by day 14 (Yates et al. 1999).

In the present work, hyphae of *T. harzianum* were observed to coil around the hyphae of the *F. verticillioides*. At some point hyphae could enter directly or form mycelial bunches around the entry point, resulting in the shriveled pathogen hyphae killing the pathogen. More or less similar hyphal interactions were observed by Dubey (1998a, 1988b), Dubey and Patel (2001), and Ahmed et al. (1999). The establishment of antagonist in a soil or substrate and its subsequent proliferation may be an important factor in biological control of pathogens and this establishment of the antagonistic fungi also depends on the age of the inoculum used (Lewis and Papavizas 1984).

Three different maize cultivars i.e. kanchan, pioneer and sweet corn, which are moderately resistant, resistant and susceptible to *F. verticillioides* respectively, were collected from maize research station and were used for ear rot disease management studies. Each cultivar had different ranges of *F. verticillioides* infection. Seeds were treated with pure culture of conidial suspension of *T. harzianum* (Th-8) which reduced the incidence of *F. verticillioides* and increased the seed germination and vigour index to a maximum extent compared to talc powder, corn starch, paddy husk and wheat bran formulations of treatment and carbendizim treatment.

Pure culture and formulation *T. harzianum* (Th-8) were also used in the field condition as seed treatment and spray treatment alone and in combination to evaluate seed quality

parameters such as field emergence, plant height, ear rot disease incidence, grain yield and level of fumonisins production for three consecutive years.

Our results revealed that *T. harzianum* significantly improved field emergence, plant height, grain yield and reduced the level of fumonisins in maize grains and controlled ear rot disease. Seed treatment followed by spray treatment was more effective than seed and spray treatment alone. Similar observations were made in laboratory condition with the same biological agents, which reduced seed-borne infection of *F. verticillioides* and increased germination and seedling vigour. There is no single replacement for chemical fumigants and fungicides that will provide similar efficacy in controlling Fusarial diseases. The present study reveals *F. verticillioides* as seed-borne; these pathogens have also been proved earlier as soil-borne and have the capacity to proliferate and sustain well in the soil (Martyn and Miller 1996). Based on the results obtained, it can be concluded that seed treatment with the microbial agents followed by foliar spray at effective concentration is efficient for enhancing the yield attributing characters. The mode of treatment, apart from reducing the incidence of diseases (relied on natural incidence) under field conditions, also improved the seed quality and also there was an enhancement in the seed yield as 1000 seed weight as assessed along with other yield attributing characters of the crop. Cook and Baker (1993) applied the same strategy and successfully used *T. harzianum* as seed treatment to improve strands of corn, beans and other vegetable crops. Kleifeld and Chet (1992) proposed that *T. harzianum* can enhance seed germination and promote plant growth and flower production. *T. harzianum* enhanced germination and growth for bean, radish, tomato, pepper, and cucumber plants following inoculation with the fungus.

A combined application of different formulations to seed and foliage was the most effective method for control of disease in the field. Possibly both rhizosphere and phyllosphere populations of *T. harzianum* helped to control disease.

The formulations of *T. harzianum* not only controlled ear rot disease, they also increased the yield when used as seed + spray treatment more effectively than carbendizim and reduced the fumonisins level in maize cultivars.

Lower disease incidence and resultant yield increase in seeds treated with microbial agents might be attributed to rapid multiplication of antagonists in the soil and its colonization in the roots of seedlings, thereby preventing the establishment of pathogens in the rhizosphere. The overall performance of *T. harzianum* in greenhouse and field conditions were consistent during the study. In all three years of the study, seed treatment followed by foliar applications showed a considerable reduction in the disease epidemics and fumonisins production compared to untreated seeds.

The biocontrol agents apart from increasing the defense responses of the plants have also been shown to increase the seed germination and seedling growth. In the present study *T. harzianum* increased the seed germination and seedling vigour over the untreated control in pioneer, kanchan and sweet corn cultivars, respectively. These results are in line with some of the other studies carried out earlier. Raju et al. (1999) showed that germination increased when sorghum seeds were treated with pure culture and talc formulation of *T. harzianum*.

Increased growth of plants by timely application of *T. harzianum* might be due to the elimination of minor pathogens in the rhizosphere (Harman and Hadar 1983). According to Windham et al. (1986) the effect on the growth might be the result of production of growth regulators by *Trichoderma* spp. Seed treatment and spraying spore suspensions of *T. viride* on growing plants of linseed controlled *Alternaria linicola*. Moderate control of *A. alternata* and *A. brassicae* was also achieved with *Echinochoclium nigrum* on rapeseed (Mercer et al. 1991).

Seeds treated with biological agents under field conditions, consisting of four replications for a three consecutive years, were analysed for fumonisins production; the amounts of fumonisin were significantly reduced throughout the treatments especially seeds treated with pure culture *T. harzianum* as seed + spray treatment, compared with other seed treatments and untreated control showed a high accumulation of fumonisin. These experiments indicated that *T. harzianum* prevented fumonisin produced by reducing the accumulation of *F. verticillioides* in maize.

The present results provide the first evidence for activity of a species *T. harzianum* as a suppressor of fumonisin synthesis. These results also support earlier reports that certain strains of *Trichoderma* sp. inhibit *F. verticillioides* growth (Calistru et al. 1997). The present study has shown that *T. harzianum* is ecofriendly and much more effective against *F. verticillioides* and Fumonisins and can be used as an alternative to fungicides to control toxigenic moulds.

References

Abdul Baki AA, Anderson JP. 1973. Vigour determination in soybean seeds by multiple criteria. Crop Sci. 13:630–633.

Ahmed AS, Sanchez CP, Egea C, Candela ME. 1999. Evaluation of *Trichoderma harzianum* for controlling root rot caused by *Phytophthora capsici* in pepper plants. Plant Pathol. 48:58–65.

Biswas KK, Das ND, Mandal UK. 1999. Management of pigeonpea wilt caused by *Fusarium oxysporum f. sp. udum* in dryland Alfisol. Ind J Dry Land Agric. Res Dev. 14(2):77–80.

Calistru C, McLean M, Berjak P. 1997. *In vitro* studies on the potential for biological control of *Aspergillus flavus* and *Fusarium moniliforme* by *Trichoderma* species: a study of the production of extracellular metabolites by *Trichoderma* species. Mycopathologia. 137:115–124.

Cook RJ, Baker KF. 1993. The nature and practice of biological control of plant pathogens. St. Paul (MN): Ann. Phytopathol. Soc. p. 593.

Domsch KH, Gams W, Anderson TH. 1980. Compendium of soil fungi. New York: Academic Press. *Trichoderma* pers, ex. 1821. p. 368–377.

Dubey SC. 1998a. Evaluation of fungal antagonists of *Thanatephorus cucumeris* causing web blight. J Mycol Plant Pathol. 28(1):15–18.

Dubey SC. 1998b. Evaluation of different fungal antagonists, plant extracts and oil cakes against *Thanatephorus cucumeris* causing banded blight of rice. J Mycol Plant Pathol. 28:266–269.

Dubey SC, Patel B. 2001. Evaluation of fungal antagonists against *Thanatephorus cucumeris* causing web blight of urd and mung bean. Indian Phytopathol. 54(4):206–209.

Elad YI, Chet I, Katan J. 1980. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology. 70:119–121.

Gelderblom WCA, Jaskiewicz K, Marasas WFO, Thiel PG, Horak RM, Vleggaar R, Kriek NPJ. 1988. Fumonisins novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl Environ. Microbiol. 54:1806–1811.

Gomez KA, Gomez AA. 1984. Statistical procedures for agricultural research. New York: John Wiley and Sons. p. 680.

Harman GE, Hadar Y. 1983. Biological control of *Pythium* species. Seed Sci Technol. 11:893–906.

ISTA. 2003. International Rules for seed testing. Seed Sci Technol. 24:28–42.

Jagadeesh Kumar CK, Niranjana SR, Prakash HS, Shetty HS. 1995. Use of antagonists to control seed-borne pathogens of maize (*Zea mays* L.). Ind J Microbial Ecol. 5:125–136.

Kleifeld O, Chet I. 1992. *Trichoderma harzianum*-interaction with plants and effect on growth response. Plant Soil. 144:267–272.

Lewis JA, Papavizas GC. 1984. A new approach to stimulate population proliferation of *Trichoderma* species and other potential biocontrol fungi introduced into natural soils. Phytopathology. 74:1240–1244.

Martha RK. 1992. Influence of some physico-chemical factors on the germination and growth of biotype of Trichoderma harzianum and Gliocladium virens, M.Sc. dissertation. Mohanpur: B.C.K.V.

Martyn RD, Miller ME. 1996. Monosporascus Root rot and vine decline. An emerging disease of melons worldwide. Plant Dis. 80(7):716–725.

McQuilken MP, Halmer P, Rhodes DJ. 1998. Application of microorganisms to seeds. Microbiol./ Rev Can Microbiol. 44(2):162–167.

Mercer JC, Martyn RD, Miller ME, Bruton BD. 1991. Role of *Monosporascus cannonballus* and other fungi in a root rot/vine decline disease of muskmelon. Plant Dis. 75:667–673.

Miller JD. 1999. Factors affecting the occurrence of fumonisin in corn. Abstract of Papers: International Conference on the Toxicology of Fumonisin, 1999, June 28–30, Arlington. p. 21.

Nelson PE, Desjardins AE, Plattner RD. 1993. Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry and significance. Annu Rev Phytopathol. 31:233–252.

Niranjan Raj S, Chaluvaraju G, Amruthesh KN, Shetty HS, Reddy MS, Kloepper JW. 2003. Induction of growth promotion and resistance against downy mildew on Pearl Millet (*Pennisetum glaucum*) by Rhizobacteria. Plant Dis. 87(4):380–384.

Perira MR, Roberts WD. 1991. Alginate and cornstarch mycelial formulations of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*. J Econ Entomol. 84:1657–1661.

Pratibanda AK, Upadhyay JP, Mukhyopadhyay AN. 2002. Efficacy of *Trichoderma harzianum* Rifai alone or in combination with fungicides against *Sclerotium* wilt of groundnut. J Biol Control. 16(1):57–63.

Prelusky DB, Trenholm HL, Savard ME. 1994. Pharmacokinetic fate of 14C-labelled fumonisin B1 in swine. Nat Toxins. 2:73–80.

Rabindran R, Vidyasekaran P. 1996. Development of a formulation of *Pseudomonas fluorescens* PfALR2 for management of rice sheath blight. Crop Protect. 15:715–721.

Raju NS, Niranjana SR, Janardhana GR, Prakash HS, Shetty HS, Mathur SB. 1999. Improvement of seed quality and field emergence of Fusarium moniliforme infected sorghum seeds using biological agents. J Sci Food and Agriculture. 79:206–212.

Reid LM, Hamilton RI, Mather DE. 1996. Screening maize for resistance to giberella ear rot. Agriculture and Agri-Food Canada, Ottawa, Ont. Technical Bulletin 1996-5E. New York: John Wiley and Sons. p. 40.

Roy AK. 1977. Parasitic activity of *Trichoderma viride* in sheath blight of fungus of rice. J Plant Disease Prot. 84:675–683.

Singh D, Kapur SP, Singh K. 2000. Management of citrus scab caused by *Elsinoe fawcetii*. Indian Phytopathol. 53(4):461–467.

Thiel PG, Marasas WFO, Sydenham EW, Shephard GS, Gelderblom WCA. 1992. The implications of naturally occurring levels of fumonisins in corn for human and animal health. Mycopathologia. 117:3–9.

Umesha S, Dharmesh SM, Shetty A, Krishnappa M, Shetty HS. 1998. Biocontrol of Downy mildew disease of Pearl millet using P*seudomonas fluorescens*. Crop Protect. 17(5):387–390.

Vidyasekaran P, Muthamilan M. 1995. Development of formulation of *Pseudomonas fluorescens* for control of chickpea wilt. Plant Dis. 79:782–786.

Williams J, John MC, Peter RM, Richard MC. 2003. A Selective Medium for Quantitative reisolation of *Trichoderma harzianum* from *Agaricus bisporus* Compost. Appl. Environ Microbiol. 69(7):4190–4191.

Windham MT, Elad Y, Baker R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology. 76:518–521.

Yates IE, Meredith F, Smart W, Bacon CW, Jaworski AJ. 1999. *Trichoderma viride* suppresses fumonisin B1 production by *Fusarium moniliforme*. J Food Prot. 62:1326–1332.