

Efficacy of foliar spray of *Trichoderma* isolates against *Fusarium graminearum* causing head blight of wheat

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Abstract

The bioefficacy of biocontrol agents, *Trichoderma harzianum* isolates (Th-M & Th-P) and *Trichoderma viride* isolate (Tv) was studied against *Fusarium* head blight pathogen of wheat (*Fusarium graminearum*) *in vitro* and under green house conditions (pot trial). Dual culture technique was used for testing the potential of *Trichoderma* isolates against seven isolates of *F. graminearum in vitro*. *Trichoderma harzianum* (Th-M) was found most effective in significantly reducing the radial growth of all *F. graminearum* isolates followed by *T. viride*. Foliar application of *T. harzianum* (M) and *T. viride* alone and in combination significantly reduced the disease severity as compared to control. Maximum reduction in disease severity was achieved with combined application of *T. harzianum* and *T. viride*.

Keywords: *Trichoderma*, *Fusarium graminearum*, head blight, wheat

1. Introduction

Wheat (*Triticum aestivum* L.) is the major staple food crop in India and grown in almost all states of the country. It is affected by several biotic and abiotic stresses. *Fusarium* head blight (FHB) or head scab has been emerged as one of the important wheat disease in the last century due to more rainy days and high precipitation during the time of anthesis and upward trends in growing-season temperatures in the world's major wheat-producing regions (Gaffen and Ross, 1998; IPCC, 2007). It is a devastating disease responsible for extensive yield and quality losses worldwide and induces toxicological problems in humans and animals (Ireta & Gilchrist, 1994, Champeil *et al.*, 2004). Scab or head blight is caused by 19 species of *Fusarium* (Parry *et al.*, 1995) but the dominant spp. in India are *F. graminearum* and *F. semitectum* (Mann and Nanda, 1999, Saharan *et al.*, 2003). Mycotoxins that pose risk to human and animal health may accumulate in wheat grain contaminated with *Fusarium* species (Mankeviciene *et al.* 2011, Baliukoniene *et al.* 2011). Disease control is effectively based on integrated management, including proper agronomic practices, utilization of resistant or tolerant cultivars and chemical applications. But the slow progress in developing scab-

resistant high-yielding cultivars is due to several reasons: the complexity of resistance, poor agronomic characters linked to resistance and low industrial quality of the well-known resistance sources. Large-scale application of fungicides is generally not practical in years of abundant rainfall, residue concerns and high cost.

Hence, biological control of crop disease is receiving more attention as an environmentally sound alternative to chemical pesticides. During the last decade, species of *Trichoderma* have emerged as the most powerful bio-protectants for the management of a number of plant diseases by virtue of their broad-spectrum action (Mukhopadhyay, 2005). The mycoparasite ability of *Trichoderma* species against some economically important aerial and soil borne plant pathogens (Papavizas, 1985; Elad *et al.*, 1993; Elad, 2000; Freeman *et al.*, 2004, Dubey *et al.*, 2007) allows for the development of biocontrol strategies. In the present study, *in vitro* efficacy of volatile metabolite and cell suspension of *Trichoderma* spp. / isolates alone and in combination were used as spray under greenhouse condition to evaluate their efficacy against *F. graminearum* (Fg), causal agent of head blight of wheat.

2. Materials and methods

2.1 Isolation of *Fusarium* spp. and *Trichoderma* spp. /isolates: Samples were collected from naturally infected wheat plant material of Wellington (Tamilnadu) [Nilgiri hills, 1850 m] and Himalayas, Lahaul spiti (3658 m amsl) during 2010-12. Surface sterilized seeds were planted on potato dextrose agar (PDA) as described by Nirenberg (1981) and incubated at 25±2°C for 3 to 5 days in BOD. The pure cultures were identified to species level based on conidial morphology, production of chlamydospores, growth characteristics, and colony pigmentation as described by Leslie *et al.* (2006). *Trichoderma* spp. /isolates were isolated from wheat rhizospheric soil using *Trichoderma*-selective medium (Elad *et al.*, 1981) by serial dilution technique. Cultures resembling *Trichoderma* spp. were purified by sub culturing on PDA and incubated at 25±2°C for 5 to 7 days. All isolates were identified on the basis of morphological characteristics (Booth, 1971). Three *Trichoderma* spp. /isolates (*T. harzianum* (M), *T. harzianum* (P) and *T. viride*) were used for further study.

2.2 Effect of volatile metabolites on radial growth of *F. graminearum*: The effect of volatile metabolites produced by the antagonistic microorganisms on *F. graminearum* mycelial growth was determined by the method described by Dennis and Webster (1971b). The antagonistic fungi were centrally inoculated by placing 5 mm mycelia disc taken from 3 days old culture on the PDA plate and then incubated at 25±2°C for 2 days. The top of each petri dish was replaced with bottom of the PDA plate centrally inoculated with the pathogen. Both plates were sealed together with paraffin tape and further incubated at 25±2°C for 15 days. In control, instead of antagonist 5 mm disc of sterile PDA medium was placed in plate. Four replications were maintained for each treatment. Colony diameter of the pathogen was measured at 5, 8 and 15 days after incubation and the inhibition of mycelial growth was determined. The percent growth inhibition was calculated by using the following equation (Vincent, 1947):

$$I (\%) = (C-T/C) \times 100$$

Where, I= % inhibition in radial growth of *Fusarium* spp., C= radial growth of *Fusarium* spp. in check (without antagonist), T= radial growth of *Fusarium* spp. with antagonist.

2.3 Evaluation of bioagents against FHB: In order to test the disease suppressive effects of *Trichoderma* isolates (Th-M and Tv, selected on the basis of *in vitro* performance) in reducing the head scab infection on wheat under green house conditions, a susceptible durum wheat variety PDW 291 was selected. Mass culture of *Trichoderma* and *Fusarium* spp. /isolates was raised on potato dextrose broth at 25±2°C for 2 weeks. The mycelial mat with spores were harvested from flasks, blended and filtered through 3-layered muslin cloth. Spore concentration of

Trichoderma and *Fusarium* suspension was adjusted to 1x 10⁹ cfu /ml and 1x10⁴ cfu /ml, respectively. To prepare the mixed inoculums of *Trichoderma*, both the isolates were mixed in ratio of 1:1 (v/v). Seeds were surface sterilized with 0.5% sodium hypochlorite solution for 10 min and sown in plastic pots (25 X 15 cm) in poly house on mid Dec. 2012. Spikes at early anthesis were sprayed by spore suspension of *Trichoderma* in different treatments (Th-M, Tv and Th-M+Tv). Control (untreated/inoculated check) plants were sprayed with sterile distilled water. Three replications were maintained for each treatment. After 3 days of biocontrol application, spikes of all the treatments were inoculated with pathogen by placing a tiny tuft of cotton soaked with the inoculum (1x10⁴ cfu /ml) in a floret of the middle spikelet. Optimum temperature (25-30°C) and humidity (RH > 90%) were maintained in poly house as described by Saharan *et al.*, 2003. Infected spikes and spikelets were counted at 14, 21 and 28 days after inoculation (DAI) and per cent disease severity was calculated as the proportion of scabbed spikelets / infected spike. Per cent *Fusarium*-damaged kernel (FDK) and were measured after harvesting. All the data were subjected to analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) to separate the treatments means.

3. Results and discussion

3.1 Effects of volatile metabolite on radial growth *in vitro*: Volatile metabolites produced by *Trichoderma* isolates in dual culture significantly reduced the radial growth of all the pathogen isolates as compared to control. The per cent growth inhibition by Th-M after 5 days of inoculation ranged from 5.22 (%) to 12.65 (%) in isolate Fg-W1 and Fg-D2, respectively (Fig. 1). Further, the inhibitory effect of volatile compounds increased after 8 and 15 days of inoculation. After 15 days of inoculation, the maximum growth inhibition (29.07%) was recorded in Fg-W3 whereas, it was minimum in Fg-W1 (5.70%). The volatile compounds from Th-P inhibited the growth of all the pathogenic isolates of *Fusarium* up to 5 and 8 days of inoculation (Fig. 2). After that a declining trend in growth inhibition was recorded at 15 days of inoculation. It indicate that Th-P isolate produces maximum volatile compounds upto 8 days and then the production of volatile compounds decrease as indicate by reduced growth inhibition after 15 days of inoculation. It might be due to that this isolate produces maximum volatile in its logarithmic growth phase and as nutrients get exhausted it growth is ceased resulted in less volatiles. In comparison to Th-M isolate, Tv has less inhibitory effect of *Fusarium* isolates. But it showed an increasing trend of growth inhibition after 5, 8 and 15 days of inoculation (Fig. 3). Maximum growth inhibition (19.05%) was achieved after 15 days of inoculation in Fg-D3 whereas;

it was minimum in Fg-W3 (4.44%). *Trichoderma* species are known to produce numerous volatile organic compounds (Stoppacher *et al.*, 2010) and these also play a major role in growth inhibition of the pathogen. Different isolates of *Trichoderma* have various strategies for fungal antagonism and effects on pathogen also varied. In another study, Behzad *et al.*, (2008) recorded similar results with *F. graminearum* sensitivity to the volatile inhibitors produced by *Trichoderma* isolates. Our results are in agreement with those of Saharan *et al.*, 2008 in which *Trichoderma* spp were found the most potent agents against *Fusarium* spp and produce inhibitory effects on mycelial growth of *F. graminearum* and *F. semitectum* causal agent of FHB in wheat. The major advantage of antibiosis via volatile metabolites is that the antibiotics substances produced by the antagonists may remain in vicinity of spikelet and spikes that help to check air borne pathogen inoculum without establishing actual physical contact with them. Each *Trichoderma* isolate differently interacted with the different isolates of *F. graminearum*. Th-M isolate gave the maximum growth inhibition across time interval against Fg-W1 followed by Fg-D2, Fg-W4 and Fg-D1. Whereas, Th-P was found more effective against Fg-W3. Similarly Tv isolate showed maximum inhibition against Fg-D3 followed by Fg-D1, Fg-D2 and Fg-W1. These results indicate that antagonistic isolates are not equally effective against all the isolates of pathogen. Therefore, selection of

antagonistic isolates to manage scab also depend on the prevalence of isolate at local level.

Effect of bioagents on FHB: The data presented in Table 1. indicated that plants sprayed with Th-M, Tv and mixed culture significantly ($P<0.05$) reduced the per cent disease severity and % FDK as compared to control. Application of mixed culture was significantly superior to reduced the disease severity in comparison to individual (Table 1) and also in reducing the % FDK (12.97). Th-M and Tv treated plants were statistically at par in reducing % FDK. After 14 DAI, disease severity was similar in all treatments (Fig. 4) but after 21 DAI and 28 DAI, disease severity was maximum in Tv treated plants. It shows that all biocontrol treatments were effective at 21 DAI and reduced the disease further progress. So, the repeated application of bioagents at disease susceptible stage can gave better results. *Trichoderma* spp. has been found to be effective in reducing the severity of foliar disease in wheat when compared to untreated plants (Sabatini *et al.*, 2002; Perello *et al.*, 2003; Muthomi *et al.*, 2007). This antagonistic mode of action of *Trichoderma* could be attributed through competition for space and nutrients, production of diffusible and/or volatile antibiotics, and hydrolytic enzymes like chitinase and -1,3-glucanase (Chutrakul *et al.*, 2008; Sharma *et al.*, 2009). These

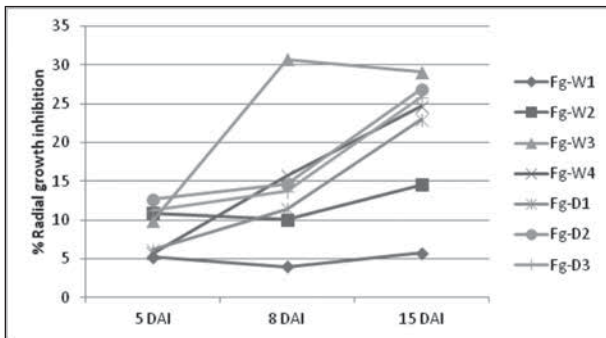


Fig 1. Effect of *Tharzianum* (M) isolates on *F.graminearum* isolates after different days of incubation (DAI)

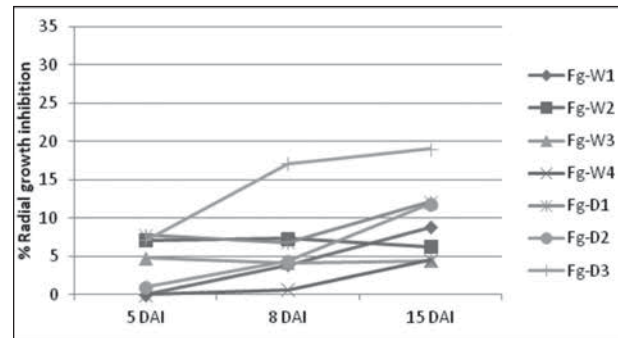


Fig 3. Effect of *T.viride* isolates on *F.graminearum* isolates after different days of incubation (DAI)

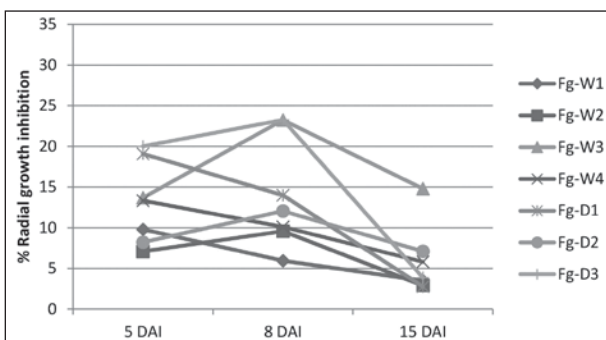


Fig 2. Effect of *Tharzianum* (P) isolates on *F.graminearum* isolates after different days of incubation (DAI)

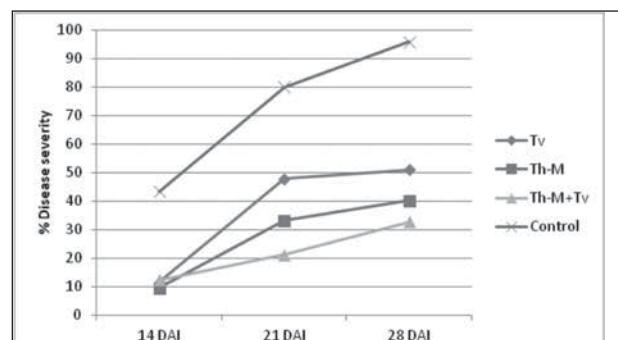


Fig 4. Effect of *Trichoderma* isolates on disease progression (% disease severity) in wheat at different intervals

hydrolytic enzymes partially degrade the pathogen cell wall and leads to its parasitization (Kubicek *et al.*, 2001). A similar inhibitory action of *Trichoderma* strains (TH1, N47 and T12) against *Pythium sp.* and *Phytophthora spp.* was reported earlier (Naseby *et al.*, 2000, Pugeg and Ian, 2006, Ezziyyani *et al.*, 2007). Biocontrol agent *T. virens* can also increase the number of healthy grains/ear as well as the yield of wheat (Sliesaravicius *et al.*, 2006; Da luz, 1998). In an earlier study, *in vitro* assays and trials in polyhouse showed that *Trichoderma* spp. were able to reduce the *F. graminearum* growth significantly (Saharan *et al.*, 2008).

Table 1. Biocontrol efficacy of *Trichoderma* on *Fusarium* head blight of wheat

Treatment	Mean AUDPC (% Disease severity)	% FDK
Th-M	407.25 ^a	21.92 ^b
Tv	553.93 ^b	21.38 ^b
Th-M+Tv	305.7 ^a	12.97 ^a
Control	1047.5 ^c	37.97 ^c

Where, Th: *Trichoderma harzianum*; Tv: *Trichoderma viride*; FDK-*Fusarium damaged kernel*, Means followed by the same letter are not significantly different

The results obtained from the foliar spray under greenhouse indicated that mixture of antagonists significantly reduced the disease. It is possible that more than one antagonistic mechanism could have been involved in the reduction of the disease. The rationale behind the use of mixed culture is that multiple strains allow the deployment of several different bio control mechanisms simultaneously. Our results support the earlier observations that a combination of biocontrol agents with different mechanisms of disease control will have an additive effect and results in enhanced disease control compared to their individual application (Guetsky *et al.*, 2002). Besides, effective control of the target pathogen over diverse set of environmental conditions could be expected if strains with same ecological requirements are included in the inoculants (Mazzola, 1998).

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