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Post Harvest Studies of Eco-friendly Managed *Fusarium graminearum* Field Infection on Wheat Seed Development and its Effect on Plant Parameters and Seed Viability

M. Rajesh, K. Prabakar and V. Valluvaparidasan

Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India

Corresponding Author: M. Rajesh, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India

ABSTRACT

In this study, the *Fusarium graminearum* infection was controlled by the biocontrol agents and zimmu leaf extract under field condition and their effects were investigated by the plant parameters and seed viability of wheat seeds. The *F. graminearum* infection on spikelet under field condition affects the shoot and root length of wheat seedlings in successive generation. Infection by *F. graminearum* may affect both the physical and physiological aspects of seed quality. In roll towel method, the maximum shoot and root length was recorded in combined application of *T. harzianum*+*P. fluorescens*+Zimmu leaf extract (T₇), which showed the shoot and root growth of 14.70 and 23.00 cm, respectively. The maximum vigour index of 3695 was recorded in the same treatment. The zimmu leaf extract, combined application with *T. harzianum* and *P. fluorescens* (T₇) and alone application (T₃) recorded the maximum seed viability of 88% which showed 37.50% increase over control. It concluded that zimmu leaf extract alone and combined with biocontrol agents effectively control the *Fusarium* head blight infection under field condition.

Key words: *Fusarium graminearum*, seed viability, plant parameter, wheat

INTRODUCTION

A first step towards the accomplishment of best possible crop yield is the use of high quality seeds (Venter, 2000). The plant pathogenic fungi invaded the seed and cause biochemical deterioration and lessen the quality of nutrients (Vijaya-Kumari and Karan, 1981). *Fusarium graminearum* cause wheat head blight was first identified in Minnesota, United States in cereal crops. *Fusarium* head blight reduces the wheat yield through floret sterility, poor seed filling and in advance stage reduces seed germination or ill-filled spikelets. Boyacioglu *et al.* (1992) reported that the *F. graminearum* infection under field condition degrades the seed quality, due to reduction of storage protein, cellulose and amylase. It may affect the both physical and physiological aspects of seed qualities, like seed size, seed weight, composition and quality. The reduced seed germination recorded in slightly infected seeds, which show uninfected embryos (Bechtel *et al.*, 1985). Sowing infected seeds may lead to widespread of plant pathogens within the crop and allows for an amplified number of initial infection sites from which the disease can spread. Wheat head blight is currently a disease of minor importance in India. Its infection on spikelet under field condition affects the shoot and root length of wheat seedlings in successive generation. There is the need to identify the effects of eco-friendly treatments under field condition

to check the *F. graminearum* infection. So, the present study is undertake to investigate the biocontrol agents and zimmu leaf extract in field condition. Their effects were measure by the plant parameters and seed viability of wheat seeds under *in vitro* condition.

MATERIALS AND METHODS

The aim of the field trial was to find out the efficacy of biocontrol agents and zimmu extract in management of seed borne *F. graminearum* under field condition. The field trial was laid out during November, 2008 to March, 2009 with wheat cultivar, HW 3094 CoW(1) at ICAR Wheat Regional Research Station, Wellington, Tamil Nadu, India.

The treatments are listed below:

- T₁ - *Trichoderma harzianum*
- T₂ - *Pseudomonas fluorescens* (Pf1)
- T₃ - Zimmu leaf extract
- T₄ - *T. harzianum*+*P. fluorescens* (Pf1)
- T₅ - *T. harzianum*+Zimmu EC formulation
- T₆ - *P. fluorescens* (Pf1)+Zimmu EC formulation
- T₇ - *T. harzianum*+*P. fluorescens* (Pf1)+Zimmu EC formulation
- T₈ - Control

The experiment was conducted in the randomized block design with a plot size of 5×3 m, adopting the recommended dosage of fertilizers and spacing. Three replications were maintained. The first spray of treatments was given at 50% flowering and second spray was given 15 days after first spray and third spray was given 10 days after second spray. At the maturity stage five heads at random were collected in each replication.

Preparation of inoculum of biocontrol agents for field spray: A loopful of 72 h old culture of *P. fluorescens* isolates was inoculated into 100 mL of sterilized KB, in 250 mL Erlenmeyer flask. The cultures were incubated for 72 h on a rotatory shaker (150 rpm) at room temperature (28±2°C). Cells were removed by centrifugation at 5000 rpm for 10 min at room temperature. Supernatant was discarded and the pellets were resuspended in 100 mL of sterile distilled water and adjusted to 10⁸ cells mL⁻¹ by dilution plate technique.

Nine millimeter disc of 7 days old culture of *T. harzianum*, isolates were inoculated into PDA. The cultures were incubated for 7 days at room temperature (28±2°C). A few mL of sterile distilled water added to the petri dish to scrap the conidial mass. The conidial loads were adjusted to 10⁸ cells mL⁻¹ by dilution plate technique. A drop of Tween 20 (polyoxyethylene sorbitan monolaurate) was added to every 250 mL of spore suspension.

Preparation of zimmu 50% EC formulation for field spray: An Emulsifiable Concentrate (EC) formulation of zimmu was developed as follows. Extracts of zimmu leaves were prepared using methanol as a solvent. Leaves (100 g) were homogenized in 200 mL methanol. The leaf homogenate was filtered through two layers of muslin cloth and the filtrate was centrifuged at 7500×g for 20 min and the clear supernatant was removed. The methanol extract was then concentrated *in vacuo* at 40°C using a Buchi EL141 Rotavapor (Flawil, Switzerland) and adjusted to a volume of 100 mL. The resultant preparation was considered as 100% extract. The leaf extract

of zimmu was formulated to 20 EC and 50 EC containing 20 and 50% (v/v) zimmu extract,, respectively, using an organic solvent (cyclohexanone) and an emulsifier (Tween-80) and stabilizer (epichlorohydrin) (Karthikeyan *et al.*, 2007).

Seed germination, root and shoot length: Germination test was conducted in between paper medium (roll towel) as per the method described by ISTA (International Seed Testing Agency). Four replicates of 100 seeds in each treatment were randomly counted and placed uniformly in roll towel method and kept in the germination room, maintained at $25\pm 2^{\circ}\text{C}$ and $95\pm 3\%$ relative humidity. On the 10th day, seedlings were evaluated and the germination was expressed as% normal seedlings.

On the final count day, ten normal seedlings were carefully removed at random from each replication and the length of root and shoot was measured separately and expressed in cm. Root length was measured from the start of the root to the tip of the primary root and shoot length from the base of the shoot to tip of the primary leaf.

Vigour index: The vigour index was calculated using the following formula and expressed as whole number (Abdul-Baki and Anderson, 1973):

$$\text{Vigour index} = \text{Germination (\%)} \times \text{Mean total length of seedlings (cm)}$$

Seed viability test: Tetrazolium chloride test was carried out to assess the viability of ten day old mould infected seeds (Lakon, 1949). The seeds were soaked in water for 16 h, after that imbibed seeds were bisected longitudinally so as to expose the embryo. The each half was placed in aqueous solution of 1.0% Tetrazolium chloride solution (2, 3, 5-Triphenyl Tetrazolium Chloride) at room temperature. Tetrazolium solution was discarded after 4 h and seeds were rinsed with water for three times. The seeds were examined under lens and evaluated for viability.

Statistical analysis: The statistical analysis of the experimental data was carried out by adopting the standard method (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The studies on the effect of *F. graminearum* infection on shoot and root length revealed that maximum shoot and root length was recorded in *T. harzianum*+*P. fluorescens* (Pf1)+Zimmu EC formulation (T_7) treated plot seeds, which showed the shoot and root growth of 14.70 and 23.00 cm, respectively Fig. 1a-b. Followed by T_6 treatment (*P. fluorescens* (Pf1)+Zimmu EC formulation) recorded 14.65 and 22.95 cm, respectively and both treatments (T_7 and T_6) were on par with each other. The *F. graminearum* infection significantly reduced the seedling vigour. The maximum reduction in seedling vigour (2860) was noticed in control. The maximum vigour index (3695) was recorded in the treatment zimmu leaf spray combined with *T. harzianum* and *P. fluorescens* (T_7). T_6 treatment (3610) recorded the maximum vigour index in wheat field trial (Table 1). The combined application of zimmu leaf extract with *T. harzianum* and *P. fluorescens* (T_7) and alone application (T_9) recorded the maximum seed viability of 88% which is 37.50% reduction over control (Table 2 and Fig. 2a-d). The mould pathogens significantly reduced the seed weight, seed size, seed germination, root length, shoot length, vigour index and seed viability of the infected seeds (Vasanth Baskar, 2007). This finding is in confirmation with the other reports (Singh and Agarwal, 1989). Maximum reduction in shoot length and root length was found in

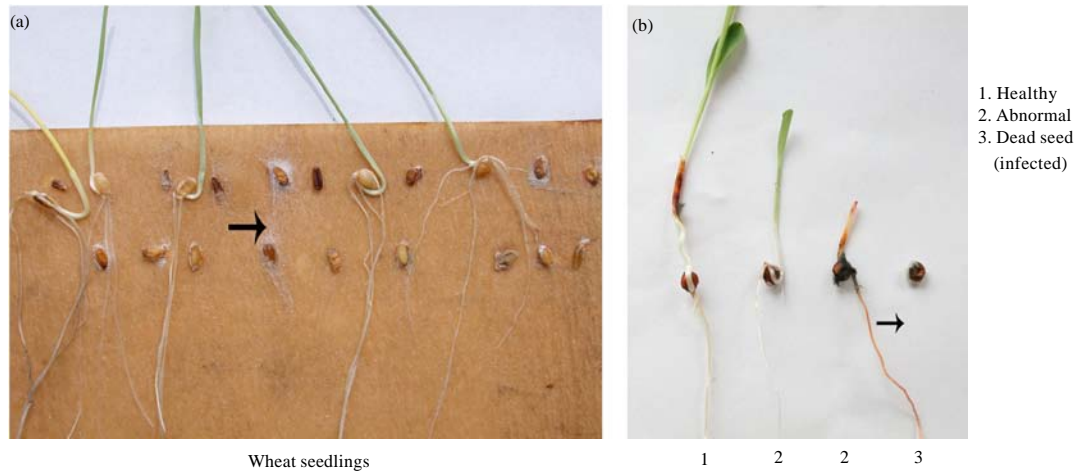


Fig. 1(a-b): Effect of Seed-borne *Fusarium graminearum* infection on wheat seeds germination, root and shoot length (Roll towel method), (a) Wheat seed germination under roll towel method and (b) Healthy, abnormal and dead seeds (infected) of wheat

Table 1: Effect of head blight infection on seed and seedling characters of wheat field trial

T. No.	Germination (%)	Shoot length (cm)		Root length (cm)		Seedling vigour index*	
		Shoot length*	Percent increase over control	Root length*	Percent increase over control	Vigour index*	Percent increase over control
T ₁	89	13.55	0.37	19.90	4.74	2977	04.09
T ₂	90	13.65	1.11	19.30	1.58	2966	03.69
T ₃	96	14.50	7.41	22.00	15.79	3504	22.52
T ₄	91	13.85	2.59	21.00	10.53	3171	10.89
T ₅	94	14.55	7.78	22.50	18.42	3483	21.77
T ₆	96	14.65	8.52	22.95	20.79	3610	26.21
T ₇	98	14.70	8.89	23.00	21.05	3695	29.18
T ₈	88	13.50	--	19.00	--	2860	--
CD (p = 0.05)	4.58	1.22		1.84		185.37	

CD: Critical deviation, *Mean of three replications, T₁: *Trichoderma harzianum*, T₂: *Pseudomonas fluorescens* (Pf1), T₃: Zimmu leaf extract, T₄: *T. harzianum*+*P. fluorescens* (Pf1), T₅: *T. harzianum*+Zimmu EC formulation, T₆: *P. fluorescens* (Pf1)+Zimmu EC formulation, T₇: *T. harzianum*+*P. fluorescens* (Pf1)+Zimmu EC formulation, T₈: Control

F. moniliforme, the greater reduction in the seedling vigour to the extent of 85.22% in *F. moniliforme* (Vasanth Baskar, 2007). Egli *et al.* (1990) reported that low values of vigour index are dependent on the number of fungi associated with seeds and poor vigour index potentially influence dry matter accumulation by plants and affects yield. Vigour of seedlings was reduced, due to infection of seeds by mould pathogens (Williams and McDonald, 1983). Prom *et al.* (2003) observed that the fungi typically cause discoloration, moulding on the grain surface and reduced seedling vigour as measured by seedling blight. Mythili (2005) reported that the culture filtrates of *A. flavus* recorded reduction in shoot length, root length and vigour index of rice seedlings. The reduction in germination and vigour index can be due to enzymes/toxins produced by the fungi and similar findings were also reported by Bhale *et al.* (1982).

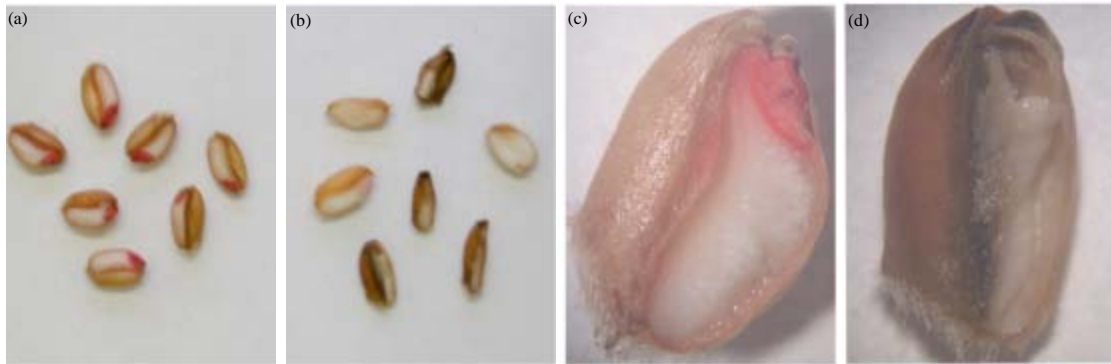


Fig. 2(a-d): Effect of Seed-borne *Fusarium graminearum* infection on viability of wheat seeds, (a) Viable seeds, (b) Non-viable seeds, (c) Viable embryo and (d) Infected embryo

Table 2: Effect of *F. graminearum* infection on viability of wheat seeds

Treatments	No. of viable seeds/25 seed	Seed viability percentage	Increase over control
<i>Trichoderma harzianum</i> (T ₁)	17	68	06.25
<i>Pseudomonas fluorescens</i> (Pf1) (T ₂)	18	72	12.50
Zimmu leaf extract (T ₃)	22	88	37.50
<i>T. harzianum</i> + <i>P. fluorescens</i> (Pf1) (T ₄)	18	72	12.50
<i>T. harzianum</i> +Zimmu EC formulation (T ₅)	21	84	31.25
<i>P. fluorescens</i> (Pf1)+Zimmu EC formulation (T ₆)	21	84	31.25
<i>T. harzianum</i> + <i>P. fluorescens</i> (Pf1)+Zimmu EC formulation (T ₇)	22	88	37.50
Control (T ₈)	16	64	--
CD (p = 0.05)	1.69		

CD: Critical deviation

The present study, the viability of seeds was reduced due to infection by *F. moniliforme* and *C. lunata*. Viability of seed was reduced by sprouting and colonization of grain by *F. semitectum*, *Curvularia* sp. and *Alternaria* sp. (Castor and Frederiksen, 1980). The viability of sorghum seed was reduced upto 100% due to severe infection by species of *Fusarium* and *Curvularia* (Rao and Williams, 1977). Narasimhan and Rangaswami (1969) reported that grain mould infection reduced the viability of sorghum seeds.

CONCLUSION

Finally this study concluded that zimmu leaf extract alone and combined with biocontrol agents effectively control the *Fusarium* head blight infection in field condition. This impact is ultimately shown in the post-harvest effect. It increases the plant parameters like, shoot length, root length and vigour index of wheat seedlings under *in vitro* condition.

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