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Harsh Vardhan Chauhan
Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Sudha Nandni
Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Pankaj Rautela
Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

RK Sahu
Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Correspondence
Harsh Vardhan Chauhan
Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Evaluation of antagonistic potential of *Trichoderma harzianum* isolates against *Fusarium Moniliformae* causing pokkah boeng of sugarcane

Harsh Vardhan Chauhan, Sudha Nandni, Pankaj Rautela and RK Sahu

Abstract
In an attempt to develop effective biocontrol system for the management of pokkah boeng caused by *Fusarium moniliformae*, five isolates of *Trichoderma harzianum* viz., Th-5, Th-9, Th-10, Th-28 and Th-32 were tested for their antagonistic potential against *F. moniliformae*. Results recorded that the strains were found effective in inhibiting the mycelial growth. Maximum percent inhibition was recorded in Th-28 (42.94%) followed by Th-32 (41.17%), Th-10 (39.21%), Th-9 (31.17%) and least in Th-5 (30.00%) as compared to control. The little difference in percent inhibition of mycelial growth indicates the difference in their efficacy against the pathogen.

Keywords: Biological control, *Fusarium moniliformae*, *Trichoderma harzianum*

Introduction
Sugarcane is a major source of sucrose (sugar), gur, khandsari etc. It is monocotyledonous, heighted grass belongs to order *Poales*, family Poaceae, sub-family *Ponicoideae*, tribe *Andropogoneae*, sub-tribe *Saccharastrae*, genus *Saccharum*, species *officinarum* [1]. It is basically a C4 plant which exploits solar energy through photosynthesis and fixes CO_2 by using C4 metabolic pathway [2]. The yield ultimately depends on the size and efficiency of this photosynthesis system [2].
Pokkah boeng disease of sugarcane was reported as minor foliar disease caused by *Fusarium moniliformae* in early 1930s. It has been noticed that the diseased plants become deficient of trace elements and few major elements simultaneously. The pol percent and sucrose percentage in juice also decline. Since this disease is spreading fast in wider areas, it appears essential to study the deterioration in sugarcane due to this disease [3]. Pokkah boeng is an emerging minor disease not only in central Uttar Pradesh but also in the whole of the Southern and Northern sugarcane growing zone of India causing reduction in the yield of sugarcane. Approximately 40.8 - 64.5% sugars can be reduced from sugarcanes infected by *Fusarium moniliforme* var. *subglutinans*, depending upon the cultivars [4]. In India the incidence and severity of Pokkah boeng disease has been reported from major sugarcane growing states like Uttar Pradesh, Uttarakhand, Maharashtra, Karnataka, Andhra Pradesh, Punjab, Haryana, Rajsthan, Assam, Tamil Nadu and Bihar etc. [5]
Biological control has attained impetus in modern era of agriculture science to curtail hazards of intensive use of toxic chemicals against pests and diseases as they pose a serious menace to human health and environment. *F. moniliforme* colony diameter alone and in dual cultures with the isolates of the *Trichoderma* species showed antagonism to *F. moniliforme* [6]. The isolates of the *Trichoderma* sp. suppressed growth of *F. moniliforme* colonies with time, increasing from 46% suppression observed on day 6 to a maximum of 91% by day 14. Different antagonists viz, *Trichoderma harzianum* and *Trichoderma viride* have been used against *Fusarium oxysporum* f.sp. *lycopersici* [7]. Under *in vitro* conditions *Trichoderma harzianum* was able to reduce the growth of *Fusarium oxysporum* to a remarkable extent [8]. *Trichoderma harzianum* was able to reduce the growth of *Fusarium solani* to a remarkable extent [9]. Two antagonistic fungi viz., *Trichoderma harzianum* and *Trichoderma viride* were evaluated for their antagonistic effect against *Fusarium oxysporum* f.sp. *ciceri in vitro* by dual culture method among which maximum inhibition was recorded in *Trichoderma harzianum* (83.33%) followed by *Trichoderma viride* (75.66%) [10].

Materials and Methods
Plant material and isolation of fungus
In kharif season of 2013, leaf samples were collected from Pokha boeng infected sugarcane plants (Co-1148) from Sugarcane Pathology block, G.B. Pant University of Agriculture and

Technology, Pantnagar. The fungus was isolated on Potato Dextrose Agar (PDA) and incubated at 28 ± 1 °C. The growing mycelium from the margin of distinct colonies was sub-cultured on fresh Petri plates containing (PDA) to obtain pure culture [11]. Purification of the resulting isolates was done using the hyphal tip or single spore technique to obtain pure cultures [12-14]. The detected isolates were then transferred into a slant of PDA and kept at 4 °C for further studies. Pure cultures of the isolated fungi were identified in accordance with the cultural properties, morphological, and microscopical characteristics of each fungus.

Screening of *Trichoderma* strains for *in-vitro* antagonism against *Fusarium moniliformae*

Different strains of *Trichoderma viz;* Th-5, Th-9, Th-10, Th-28, and Th-32 were tested *in vitro* for the antagonistic potential against test pathogen. The experiment was carried out on PDA medium using dual culture method [15].

Twenty ml of sterilized melted PDA was aseptically poured in a sterilized 85 mm diameter Petri plates and allowed to solidify 5 mm mycelial disc of *F. moniliformae* and test bio-control agents cut with the help of sterilized cork borer (with outside/inside diameter of 7/5.5) from the edge of four days old culture plates, were placed on solidified PDA in such a manner that they lie just opposite to each other (approximately 6 cm apart from each other). Inoculated Petri plates were incubated at 28±1 ^{0}C. Periodic observations on the growth of bio-control agents and the ability of bio-control agents to colonize the pathogen was recorded when the growth of the control plates completely covered the plates. The percent inhibition in growth was calculated by the following formula:

$$\text{Percent inhibition} = \frac{X - Y}{X} \times 100$$

Where,

X = colony diameter in control

Y = colony diameter in treated medium

Results and Discussion

Use of bio agents for controlling plant diseases is an age old practice in India. In the last two decades great emphasis has been given on the antagonistic organism and to assess their potential for control of plant diseases, particularly as one of the component in IPM.

In the present investigation, different strains of *Trichoderma harzianum* viz., Th-5, Th-9, Th-10, Th-28 and Th-32 were evaluated to check their efficacy against *Fusarium moniliformae* by using dual culture method. Results are presented on (Table 1 & Fig. 1). Observations were recorded when the check plate was full with colony growth i.e., 85 mm. Results showed that the strains were effective in inhibiting the mycelial growth of *Fusarium moniliformae*. Maximum percent inhibition was recorded in Th-28 (42.94%) followed by Th-32 (41.17%), Th-10 (39.21%), Th-9 (31.17%) and least in Th-5 (30.00%) as compared to control. The little difference in percent inhibition of mycelial growth indicates the difference in their efficacy against the pathogen.

However, it has been reported the antagonistic effect of the *Trichoderma harzianum* against *Fusarium* spp [6-10]. In the figure, a clear cut zone of inhibition was observed with all the strains tested against test fungus. The results thus obtained indicate a need for *in vitro* evaluation of more isolates of *Trichoderma* against the pathogen, which could lead to a better eco-friendly management of disease in future at field level also.

Table 1: *In-vitro* effect of different strains of *Trichoderma harzianum* on the radial growth of *F. moniliformae* at 25-28 ± 1 °C after 10 days

S. No.	Strains of *T. harzianum*	Radial growth (mm)	Inhibition (%)
1	Th-5	59.50	30.00
2	Th-9	58.50	31.17
3	Th-10	51.67	39.21
4	Th-28	48.50	42.94
5	Th-32	50.00	41.17
6	Control	85.0	0.00
CD at 5% 1.31 SEM ± 0.42			
G= Radial growth (mm), I= % inhibition in radial growth			

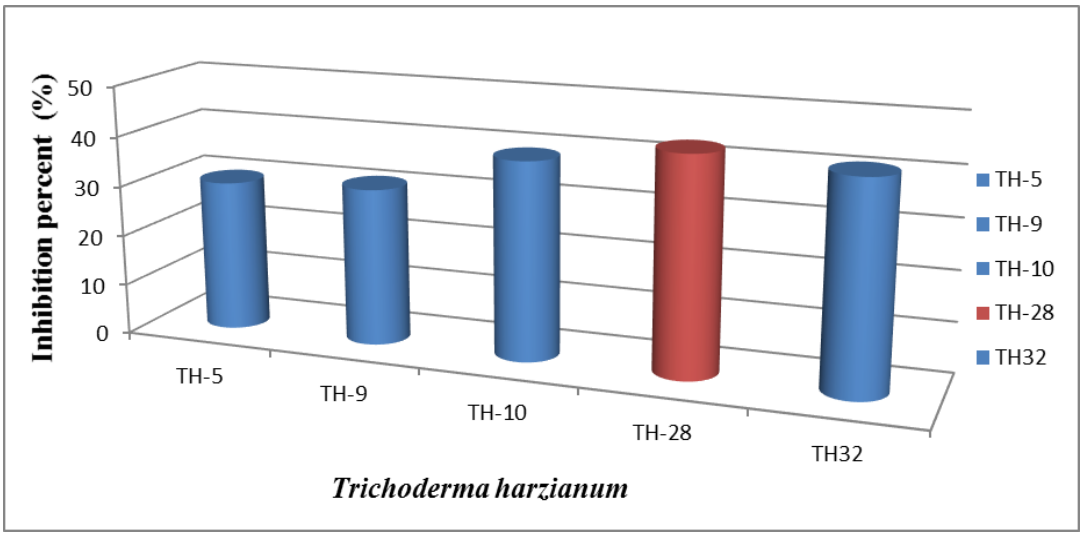

Fig 1: *In-vitro* effect of different strains of *Trichoderma harzianum* on percent ihibition of *Fusarium moniliformae* at 28 ± 1 °C after 10 days

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