J. Sci. Foundation, 8(1&2): 141-150, June-December 2010 ISSN 1728-7855

EFFECT OF *TRICHODERMA* ON SEED GERMINATION AND SEEDLING PARAMETERS OF CHILI

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ABSTRACT

T. virens IMI-392430, *T. pseudokoningii* IMI-392431, *T. harzianum* IMI-392432, *T. harzianum* IMI-392433 and *T. harzianum* IMI-392434 were evaluated for their potentiality on seed germination and seedling parameters in chili both laboratory and field conditions. Chili seeds were coated with spore suspension of each test strains of *Thichoderma* supplemented with 2 % of starch (w/v) as an adhesive. Seed germination percentages and the vigour index were significantly affected by the application of different strains of *Trichoderma*. Among the five *Trichoderma* strains, *T. harzianum* IMI-3924332 gave the highest germination percentage followed by *T. harzianum* IMI-3924333, *T. harzianum* IMI-3924334, *T. virens* IMI-392430 and *T. pseudokoningii* IMI-392431 treatment both in laboratory and field conditions, respectively while control decrease these value. Chili seeds also gave the highest vigour index values with *T. harzianum* IMI-3924332 which confirmed to better germination. Seed treatment with *T. harzianum* IMI-3924332 can be useful to enhance the germination of chili seeds as well as reduce to delayed germination. Further investigations however are required to study *in vivo* effect of *Trichoderma* strains on morphological and physiological characteristics in chili plant and fruit production.

Key word: *Trichoderma*, spore suspension, germination percentages, vigour index, chili.

INTRODUCTION

Chili (*Capsicum annuum* L.) is one of the most important spice crops in the world and grown in all seasons and areas of Bangladesh. The average yield of chili is 0.042 t ha^{-1} which is very low as compared to the yield of other chili growing countries of the world (Anonymous, 2003). Delayed and erratic germination of chili seeds is one of the reasons of low yield of chili. There are many factors responsible for the delayed and erratic germination of chili seeds. Among the various factors diseases are predominant. Fungal diseases play a vital role in reducing the germination of chili. Water imbibitions are first step in the seed germination. But crop field may lack adequate moisture content for the same, so poor and delayed germination occurs. To combat this, farmer pre soak the seed in plain water for a few hours. But this may cause seed damage in more than one ways. Of them, major one is that, excess water may be trapped in the area of embryonic axis, nodal zone and cotyledons. This leads to suffocation, resulting in delayed and poor germination as well as weak seedling growth (Heydecker, 1977). Delayed and erratic germination create problems with fertilizer utilization, post emergence weed control, and uniform harvesting (Standifer *et al*., 1989). In recent times various seed quality enhancement treatments are given to the seeds as a pre-sowing treatment. The hard seed coat of chili is also a major physiological constraint to uniform stand establishment and performance.

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Trichoderma genus is often the predominant components of the mycoflora in soils of various ecosystems, such as agricultural fields, prairie, forest, salt marshes and deserts, in all climatic zones (Harman, 2000a; Smith, 1995). It has capacity of controlling some plant and crop disease (Samuels, 1996). *Trichoderma* spp. on roots increases uptake of nitrates and other ions. And many also increase uptake of various toxic metals and metalloids (Harman, 2000b). *Trichoderma* strains are always associated with plant roots and root ecosystems. Some authors have defined *Trichoderma* strains as plant symbiont opportunistic avirulent organisms, able to colonize plant roots by mechanisms similar to those of mycorrhizal fungi and to produce compounds that stimulate growth and plant defense mechanisms (Harman *et al.*, 2004). Root colonization by *Trichoderma* strains frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (Arora *et al.*, 1992). Crop productivity in fields can increase up to 30 % after the addition of *Trichoderma hamatum* or *Trichoderma koningii* (Benítez *et al.*, 2004). In experiments carried out in greenhouses, there was also a considerable yield increase when plant seeds were previously treated with spores from *Trichoderma* (Chet *et al.*, 1997). The same increase was observed when seeds were separated from *Trichoderma* by a cellophane membrane, which indicates that *Trichoderma* produces growth factors that increased the rate of seed germination (Benítez *et al.*, 1998). *Trichoderma* also compete with other microorganisms; for example, they compete for key exudates from seeds that stimulate the germination of propagules of plant-pathogenic fungi in soil (Howell, 2002) and more generally, compete with soil microorganisms for nutrients and/or space (Elad, 1996). *Trichoderma* strains that produce cytokinin-like molecules, e.g. zeatyn and gibberellin GA3 or GA3-related have been recently detected. The controlled production of these compounds could improve biofertilization (Osiewacz, 2002). Together with the synthesis or stimulation of phytohormone production, most *Trichoderma* strains acidify their surrounding environment by secreting organic acids, such as gluconic, citric or fumaric acid (Gómez-Alarcón and de la Torre, 1994). These organic acids result from the metabolism of other carbon sources, mainly glucose, and, in turn, are able to solubilize phosphates, micronutrients and mineral cautions including iron, manganese and magnesium (Harman *et al.*, 2004). Moreover, recent studies have indicated that these fungi also induce localized or systemic resistance systems in plants (Yedidia *et al.*, 1999; Howell, 2003). Thus, the variety of effects indicates that these beneficial fungi have multiple modes of action. Therefore, this study was design to find out the effect of different *Trichoderma* strains on the germination percentage rate and seedling parameters of chili seeds both in laboratory and field conditions.

MATERIALS AND METHODS

Sources of *Trichoderma* strains

Five *Trichoderma* strains viz. *T. virens* IMI-392430, *T. pseudokoningii* IMI-392431 and *T. harzianum* IMI-392432, *T. harzianum* IMI-392433 and *T. harzianum* IMI-392434 were used in this study which was collected from Biotechnology and Microbiology Laboratory, Department of Botany, Rajshahi University, Bangladesh. These strains were isolated and identified from decomposed garbage and soil by Rahman (2009) and were verified by CABI Bioscience, Surrey, U.K.

Spore suspension preparation of *Trichoderma* strains

Mycelial disc (1.2 cm diam.) of *Trichoderma* strains were obtained from 4-5 days-old culture and transferred to 50 ml PDA in a 250-ml conical flask separately incubated at 28 ºC for 4-5 days. At the end of the incubation period 30 ml of sterile distilled water was added to each culture flask and the flasks were shaken at 50 rpm for 30 min in an orbital shaker. Then the content of each conical flask was filtrate through sterile muslin cloth. The filtrate with the spores was collected and a concentration of spore suspension was adjusted to 5×10^5conidia/ml by use of a hemacytometer under a light microscope.

Seed selection and treatment

The seed of chili Varity Bogra Local was used and collected from Spice Research Centre, Bogra, Bangladesh. The chili seeds were one year old and had been stored at 5°C. Standard germination of the seeds was 98 %. Seeds with no cracks or other visible deformations were selected and surface sterilized for 10 minutes with1 % sodium hypochlorite solution. Seeds were then rinsed three times with sterile distilled water and air dried. A seed coating was prepared from spore suspension supplemented with 2% of starch (w/v) as an adhesive. Dry chili seeds were dipped in seed coating suspension (5×10^5spores/ml) for each *Trichoderma* strains for 1-2 minutes. For untreated control seeds were dipped in 2% starch suspension and for water control seeds were dipped in sterilized distilled water. Seeds were air dried inside the laminar air flow hood. For *in vitro* experiment, treated seeds were placed in Petri plate's lines with two layers of Whatman filter paper soaked in sterilized distilled water and incubated at 25 °C under dark condition and for field experiment, treated seeds were sown separately in pot soils where the soil was previously inoculated with respective *Trichoderma* strains (5×10^5spore/ml) and for control treatment, treated seeds (treated with 2% starch and water) were sowing un inoculated soil in pot. At least ten seeds were sown in each pot. Seed germination percentages and vigour index was recorded after 3 to 8 days. Vigour index for each treatment was determined using the following formula developed by Abdul-Baki and Anderson (1973).

Vigour index = [Mean of root length (cm) + Mean of shoot length (cm)] × percentages of seed germination.

Collection and preparation of soil for field experiment

For field experiment soil was collected from the Field Laboratory of Plant Pathology Division, BARI, Gazipur and sterilized with Formaldehyde (Formalin: Water; 1:5 V/v). After 30 days of sterilization, soils were put in the earth pot of 12 inches height and 8 inches wide. For minimize losses of excess water 2 cm hole was made from the bottom of the pot.

Experimental design and data analysis

All experiments were established as a randomized block design with four replicates and ten chili seeds were used in each replicates. Data on germination percentages and vigour index were recorded after 3 to 8 days and statistically analyzed with the help of computer package program SPSS (SPSS Inc., Chicago, IL, USA) and also tested by DMRT.

RESULTS AND DISCUSSION

The effect of five *Trichoderm* strains on seed germination and seedling parameters of chili both in laboratory and field conditions the results are presented in Fig 1-8. Statistical analysis of figure showed significant differences in treatments at $P \leq 0.05$ levels. Results showed that all *Trichoderma* strains were found effective to enhance the germination percentage compared to control. However among the five *Trichoderma* strains, *T. harzianum* IMI 392432 exhibited significantly enhancement of germination percentage in chili seeds both in laboratory and field conditions followed by *T. harzianum* IMI 392433, *T. harzianum* IMI 392434, *T. virens* IMI 392430 and *T. pseudokoningii* IMI 392431 (Fig 1 and 5), while control (treated with 2% starch and water) significantly decreased these values. This strains also showed earliest highest seed germination (100%) at five and six days compared to the control both laboratory and field conditions, respectively. In controls (treated with 2% starch and water), both laboratory and field conditions showed worst germination percentage and vigour index.

Some landmarks along the way include the discoveries that these fungi frequently increase plant growth and productivity (Harman, 2006; Manju and Mall, 2008). In this study, five *Trichoderma* strains gave early germination as well as highest germination percentage which have also been reported by many workers in different plants (Hanson, 2000; Mishra and Sinha, 2000; Oyarbide *et al*., 2001) and numerous other species such as *T. longipile* and *T. tomentosum* have been shown to promote plant growth (Rabeendran *et al*., 2000).Studies have been confirmed in case of *T. harzianum* and *T. viridi* to enhanced seed germination root and shoot length (Dubey *et al*., 2007) as well as increasing the frequency of healthy plants, and boosting yield (Rojoa *et al*., 2007). In a similar study Chaur-Tsuen Lo and Chien-Yih Lin (2002) screened *Trichoderma* strains on plant growth and root growth of bitter gourd, loofah and cucumber and noted that *Trichoderma* strains significantly increased of 26 to 61 % in seedling height, 85-209 % in root exploration, 27-38% in leaf area and 38 to 62 % in root dry weight after 15 days of sowing. Methanol extract of *T. harzianum* and *T. viridi* significantly improved various growth parameters of okra (Prasad and Anes, 2008). Vigour index (VI) was also significantly affected by the application of different *Trichoderma* strains both in laboratory and field conditions (Fig 4 and 8). The results related to vigour index showed similar differences as in germination percentages. Seed treatment with *Trichoderma* strains increased vigour index compared to control. The highest VI values were recorded both in laboratory and field conditions when the chili seed were treated with *T. harzianum* IMI 392432. The lowest vigour index was recorded in control. Mukhtar (2008) investigated that seed treatment with *T. harzianum* gave the highest germination index in okra and *T. harzianum* can be useful to enhance the germination percentage as well as reduce lose due to delayed germination of okra seeds. Begum *et al.* (2010) were evaluated five *Trichoderma* strains to assay their efficacy in suppressing *Alternaria* fruit rot disease of chili and promoting chili plant growth and yield and observed that application of *T. harzianum* IMI 392432 significantly suppressed the disease and improved highest seed germination percentage, vigour index, growth and yield. Other investigators have also reported that seeds pretreated with *T. viride*, *T. harzianum* and *T. pseudokoningii* inoculant extracts, showed the increased seed germination rates, seedling vigour and reduced the incidence of seed-borne fungal pathogens compared to control (Zheng and Shetty, 2000; Bharath *et al*., 2006). The present study concludes that *Trichoderma* species have potential to enhance the germination in chili seeds which can be useful to enhance the germination percentage of chili seeds besides reducing loses due to delayed germination. Further investigations are required to study *in vivo*, effects of these fungi on the morphological and physiological characteristics in chili plant and fruit production.

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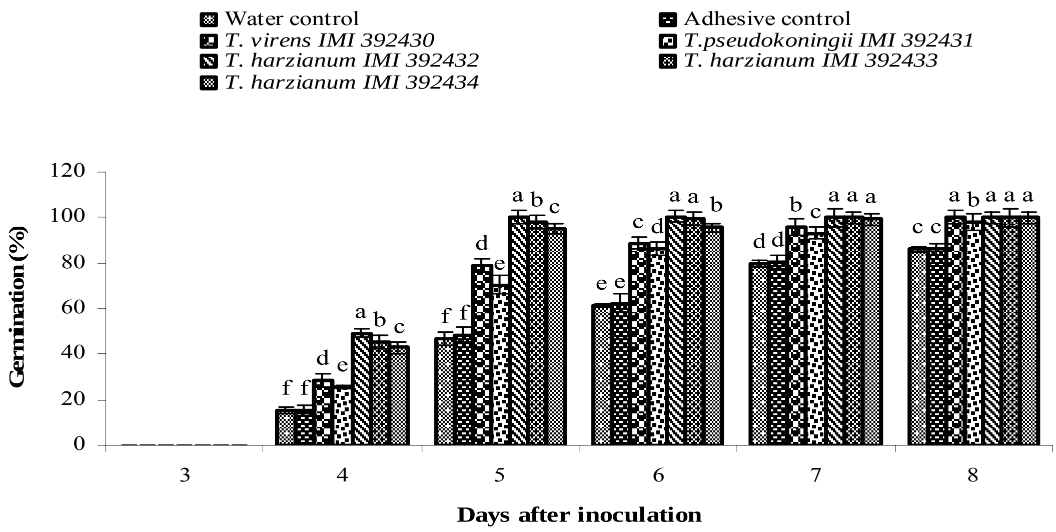

Fig 1. Effect of seed treatment with *Trichoderma* strains on the germination percentage of chili seeds in *in vitro* condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

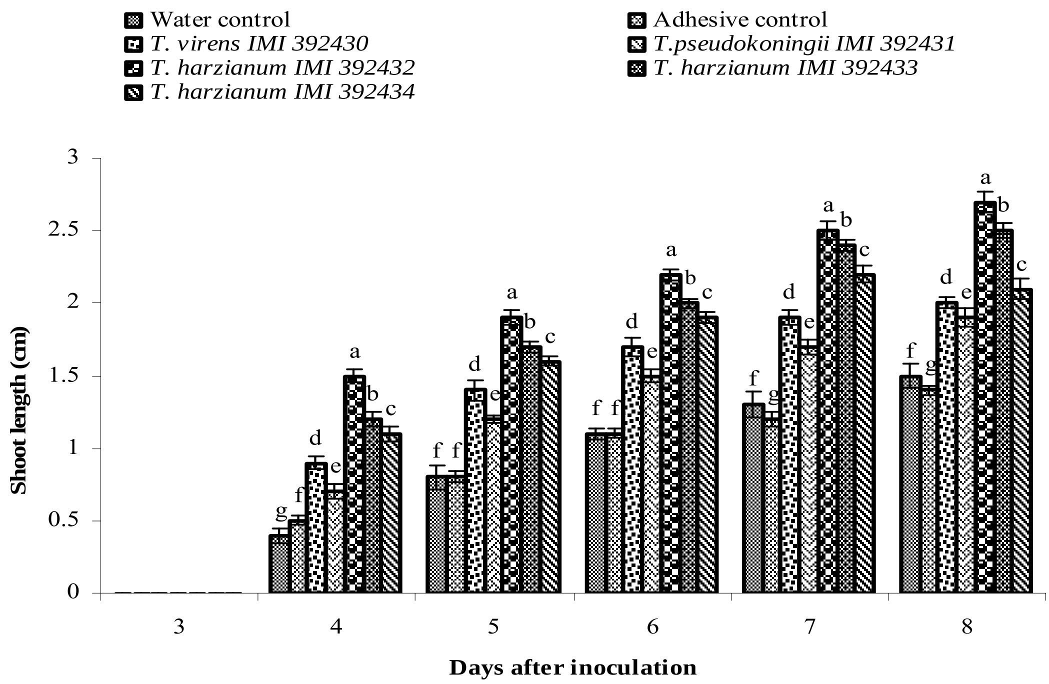

Fig 2. Effect of seed treatment with *Trichoderma* strains on shoot length of chili seeds in *in vitro* condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

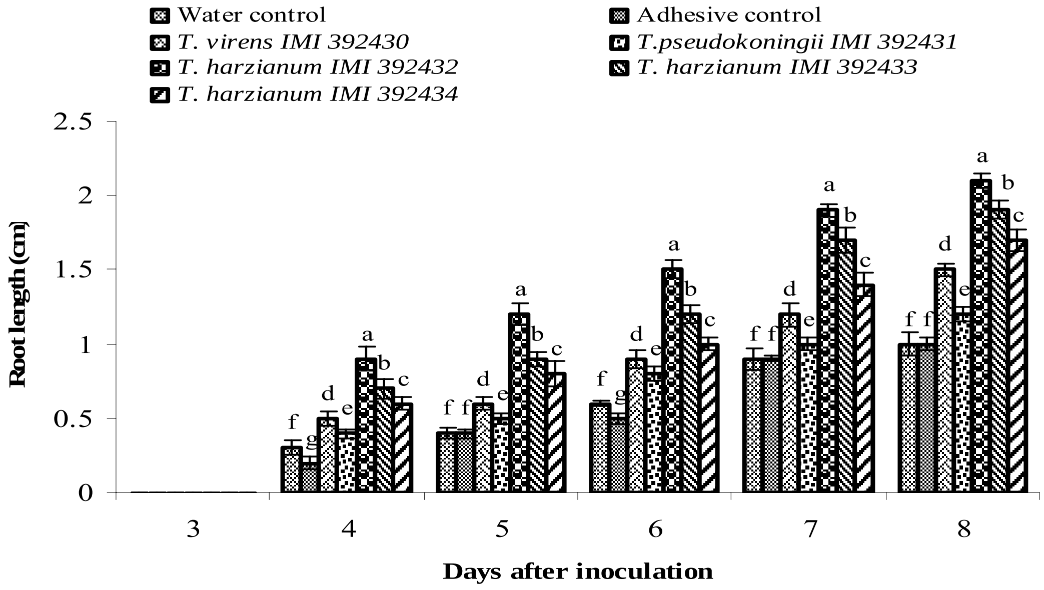

Fig 3. Effect of seed treatment with *Trichoderma* strains on root length of chili seeds in *in vitro* condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

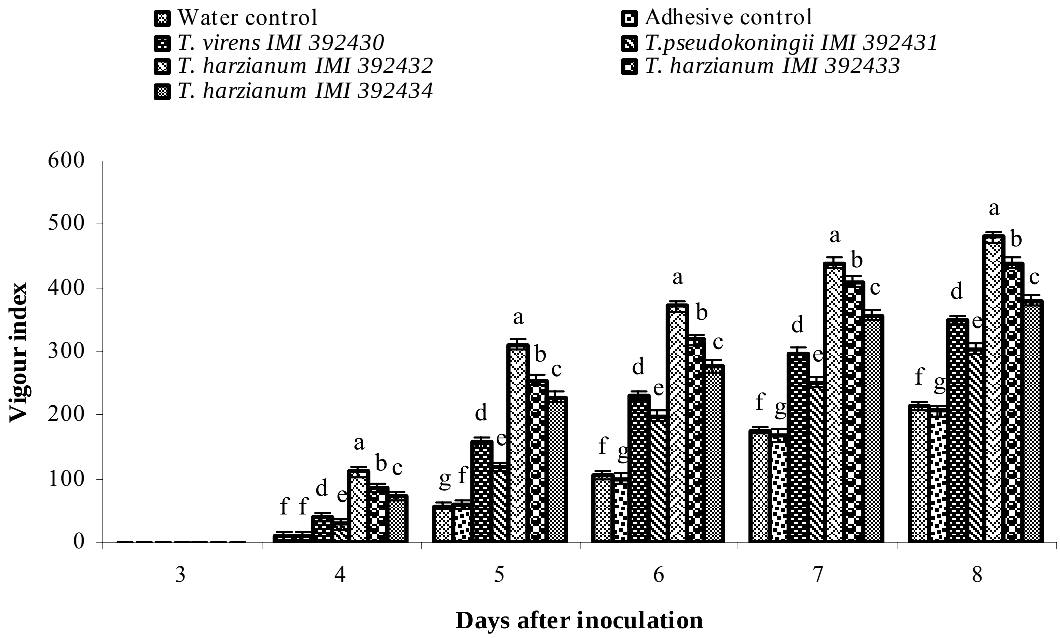

Fig 4. Effect of seed treatment with *Trichoderma* strains on vigour index of chili seeds in *in vitro* condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

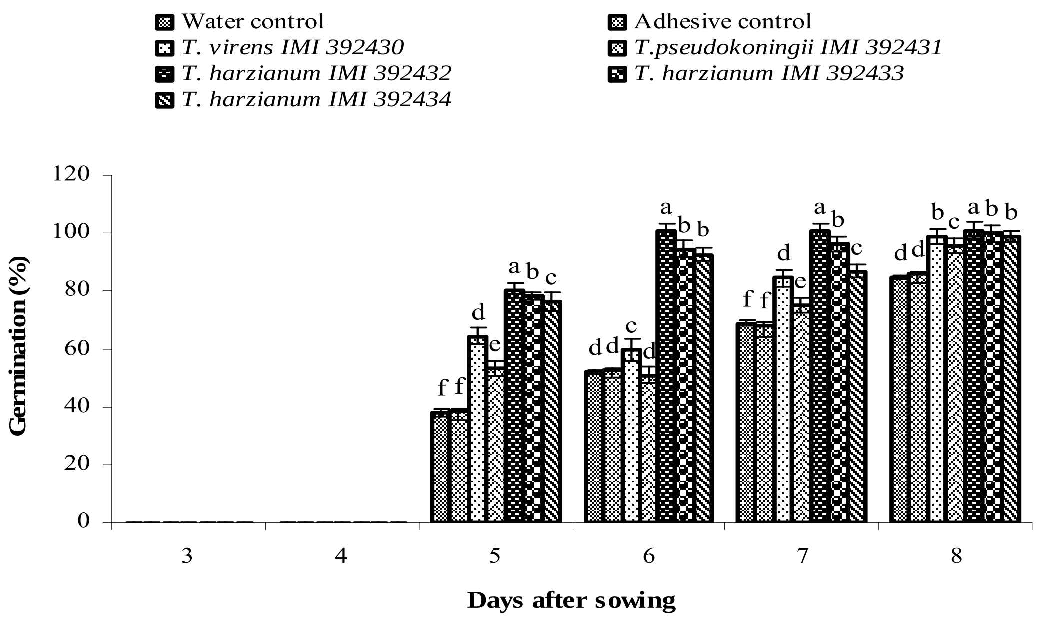

Fig 5. Effect of seed treatment with *Trichoderma* strains on the germination percentage of chili seeds in field condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

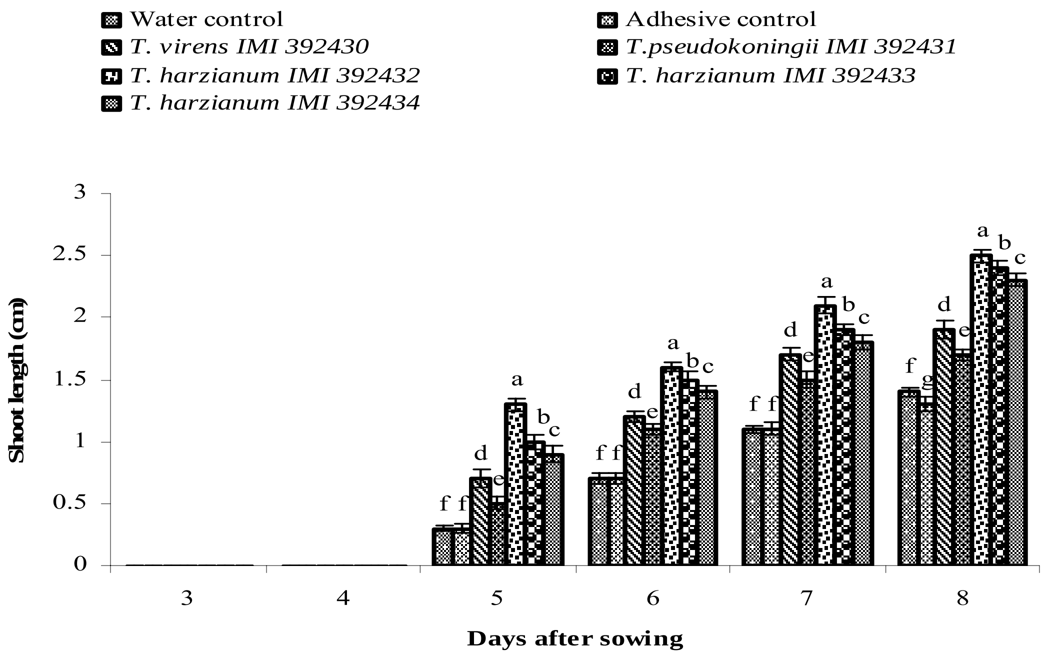

Fig 6. Effect of seed treatment with *Trichoderma* strains on the shoot length of chili seeds in field condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

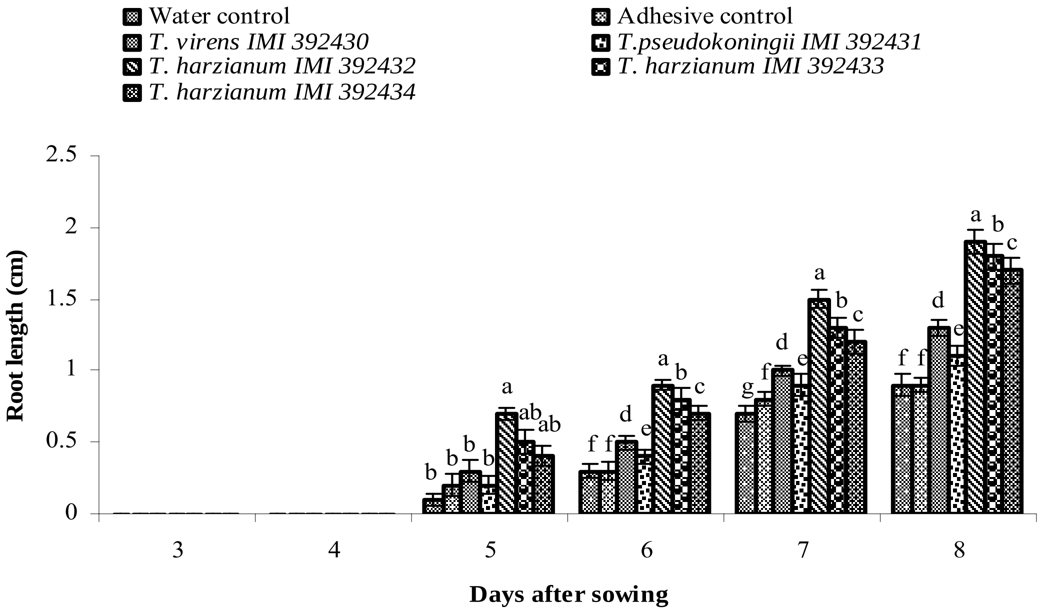

Fig 7. Effect of seed treatment with *Trichoderma* strains on the root length of chili seeds in field condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

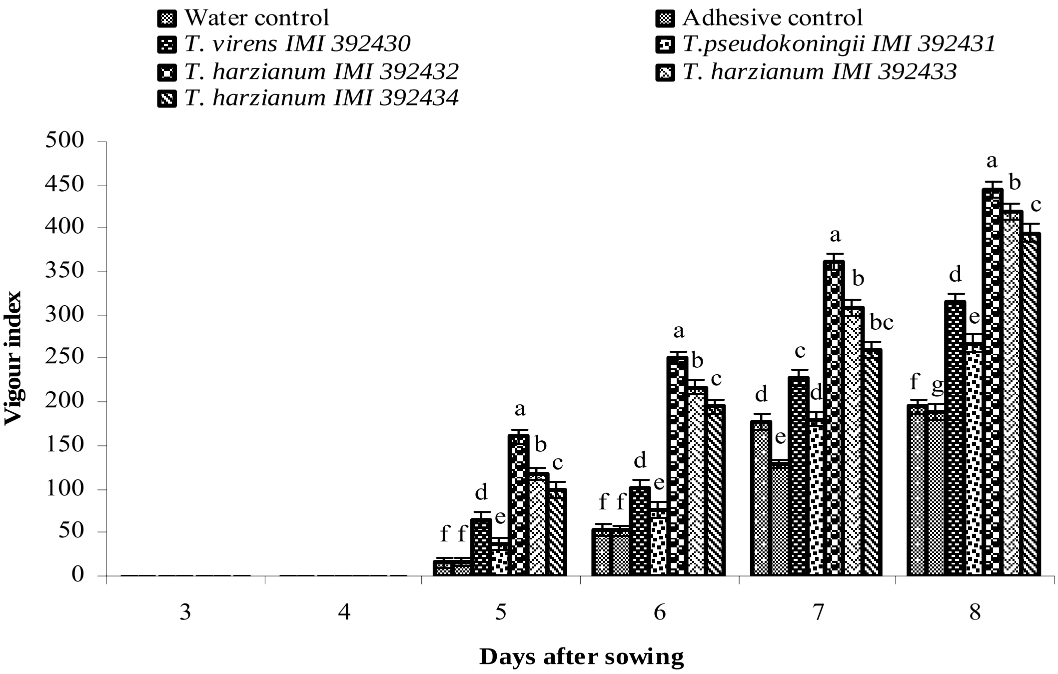

Fig 8. Effect of seed treatment with *Trichoderma* strains on the vigour index of chili seeds in field condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.