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Effect of culture filtrates of *Trichoderma* on seed germination and seedling growth in chili

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Abstract

Five *Trichoderma* strains namely; *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 of chili both in laboratory and field conditions. Chili (*Capsicum annuum* L.) seeds were coated with culture filtrates of each test strains of *Trichoderma* supplemented with 2 % of starch (w/v) as an adhesive. For laboratory experiment, ten treated seeds were placed in each Petri plate and incubated at 25°C under dark condition and for field experiment pot trial experiment was conducted and ten treated seeds were sowing in each pot. All experiments were established as a randomized block design with four replications. Germination percentages of treated seeds were recorded after 3 to 8 days. Seed germination percentages and the vigour index were significantly ($P \leq 0.05$) affected by the application culture filtrates of different *Trichoderma* strains. Among the five *Trichoderma* strains, *T. harzianum* IMI-3924332 culture filtrates gave the highest germination percentage and vigour index followed by *T. harzianum* IMI-3924333, *T. harzianum* IMI-3924334, *T. virens* IMI-392430 and *T. pseudokoningii* IMI-392431 both in laboratory and field conditions, respectively, while control (treated with 2% starch and water) decrease these value. Seed treatment with culture filtrates of *T. harzianum* IMI-3924332 can be useful to enhance the germination percent of chili seeds as well as reduce to delayed germination. Further investigations however are required to study *in vivo* effect of *Trichoderma* culture filtrate on morphological and physiological characteristics in chili plant and fruit production.

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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). The genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents (Benítez *et al.,* 2004). *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). Most *Trichoderma* strains produce volatile and non volatile toxic metabolites that impede colonization by antagonized microorganisms; among these metabolites, the production of harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-penthyl-α-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid and others have been described (Vey *et al.,* 2001), which provide to protect seedlings from disease (Chet *et al.,* 1997). The application of *Trichoderma* spp. has not only an antagonistic on plant pathogens but also a positive effect on plant growth and yield in some vegetable crops (Baker, 1989; Chang *et al.,* 1986; Inbar *et al.,* 1994; Poldma *et al.,* 2000). Crop productivity in fields can increase up to 30% after the addition of *Trichoderma hamatum* or *Trichoderma koningii*. In experiments carried out in green houses, 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., 2004). *Trichoderma* produce phytohormones, such as indol acetic acid (IAA) and ethylene, whose metabolic pathways have been identified (Arora, 1992; Osiewacz, 2002). *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 cations including iron, manganese and magnesium (Harman *et al.,* 2004). Thus, the variety of effects indicates that these beneficial fungi have multiple modes of action. The present study was therefore, undertaken to evaluate the effect of culture filtrates of different *Trichoderma* strains on germination percentage of chili seed 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.

Preparation of culture filtrates of Trichoderma

200 ml of Richard's solution (KNO_3: 1.0g, KH_2PO_4:0.5g, $MgSO_47H_2O$: 0.25g, glucose: 34g, trace amounts of $FeCl_3$ in 1L distilled water, pH6.5) was prepared and poured into 500 ml conical flasks and autoclaved for 15 minute at 121 °C/1.05kg/cm² pressure. Six pieces of agar discs (6 mm) were kept in a flask (with media) for each strains of *Trichoderma* with four replications. The flasks were incubated on a Gallenkamp orbital incubator at 100 rpm at 28 °C (Dennis and Webster, 1971). The culture filtrates were collected after 30 days of incubation. These were then concentrated to about 50 % using a vacuum evaporator at 38-40 °C and finally filtered by sterilized membrane filter.

Seed selection and treatment

The seed of chili Varity Bogra Local was used and collected from Spice Research Centre, Bogra, Bangladesh. The seeds were one years 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 with 1 % sodium hypochlorite solution. Seeds were then rinsed three times with sterilized distilled water and air dried. A seed coating was prepared from *Tricchoderma* culture filtrates supplemented with 2 % of starch (w/v) as additives. Dry chili seeds were dipped in culture filtrates supplemented with 2 % of starch (w/v) for each *Trichoderma* strains for 1-2 minutes. For untreated control seeds were dipped in 2 % starch solution and for water control seeds were dipped in water. Seeds were air dried in the laminar air flow hood and were placed in Petri plates lines with two layers of Whatman filter paper soaked in sterile distilled water. In each Petri plate, ten seeds were placed and incubated at 25°C under dark. In field experiment, treated seeds were sown separately in pot soils where the soil was previously inoculated with culture filtrates of *Trichoderma* strains (20 ml/pot) 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. Germination of seeds was compared with control treatment (treated with 2% starch and water). Seed germination percentages and vigor 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 Botanical garden of Rajshahi University Campus, Bangladesh 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 culture filtrates of five *Trichoderm* strains on seed germination of chili both in laboratory and field conditions the results are presented in Fig. 1 and 5. Statistical analysis of figure showed significant differences in treatments at $P \leq 0.05$ levels. Results showed that

culture filtrates of five *Trichoderma* strains were found effective to enhance the germination percentage compared to control (treated with 2% starch and water). However among the five *Trichoderma* strains, *T. harzianum* IMI 392432 culture filtrate 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 treatment (treated with 2% starch and water) both laboratory and field conditions, respectively. In controls (treated with 2% starch and water), both laboratory and field conditions showed worst germination percentage.

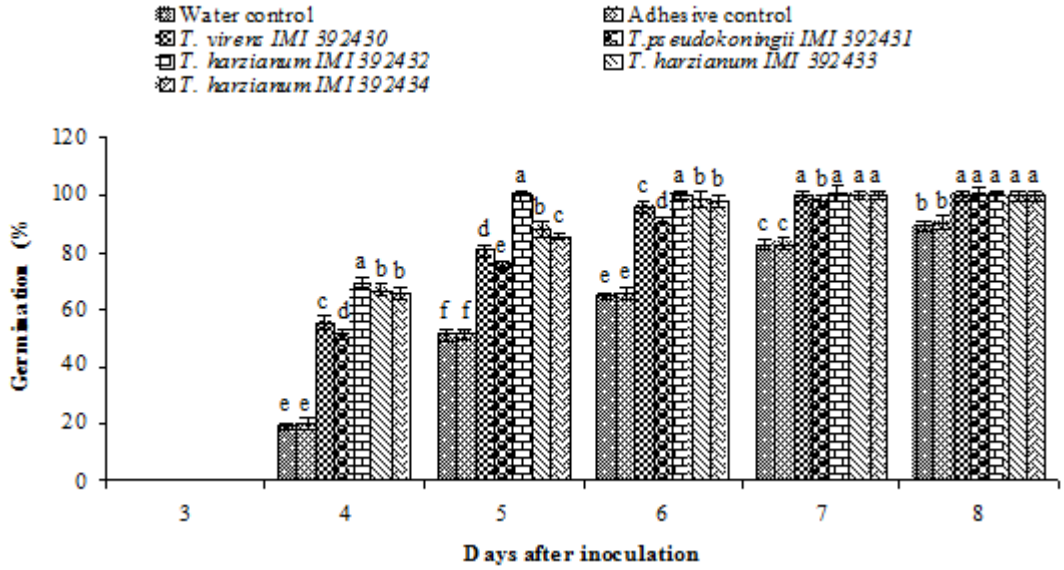

Fig. 1. Effect of seed treatment with culture filtrates of five *Trichoderma* strains on the germination percentage of chili seeds in *in vitro* condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

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 culture filtrates of *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 culture filtrates of *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).

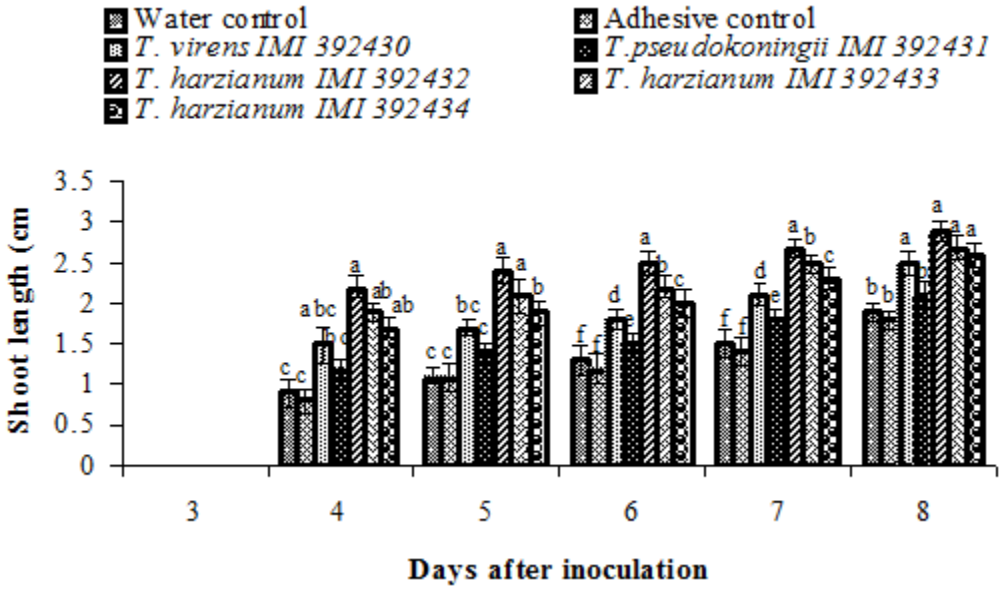

Fig. 2. Effect of seed treatment with five *Trichoderma* strains on the shoot length of chili seeds in *in vitro* condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

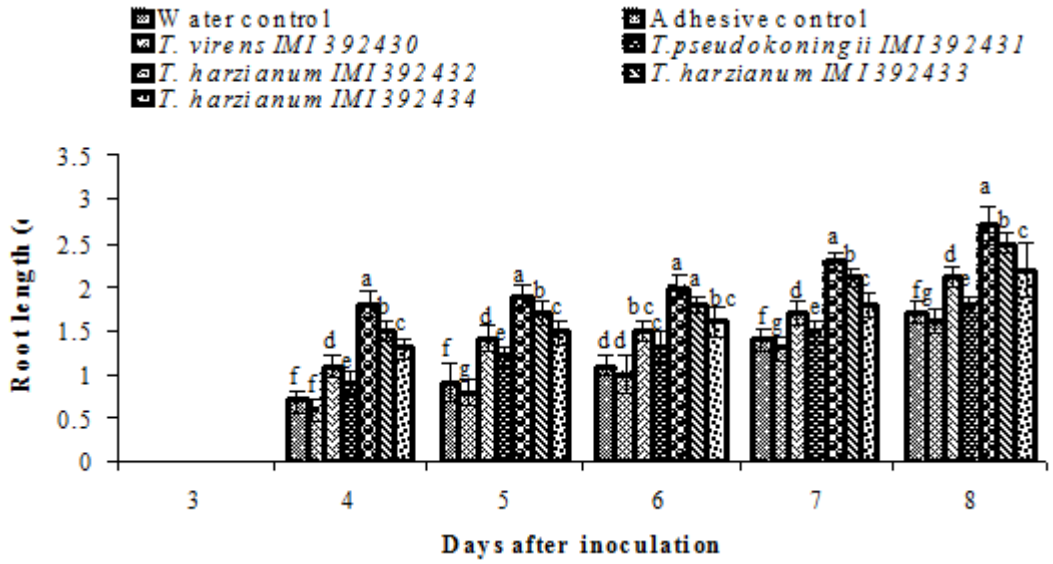

Fig. 3. Effect of seed treatment with five *Trichoderma* strains on root length of chili seeds in *in vitro* condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

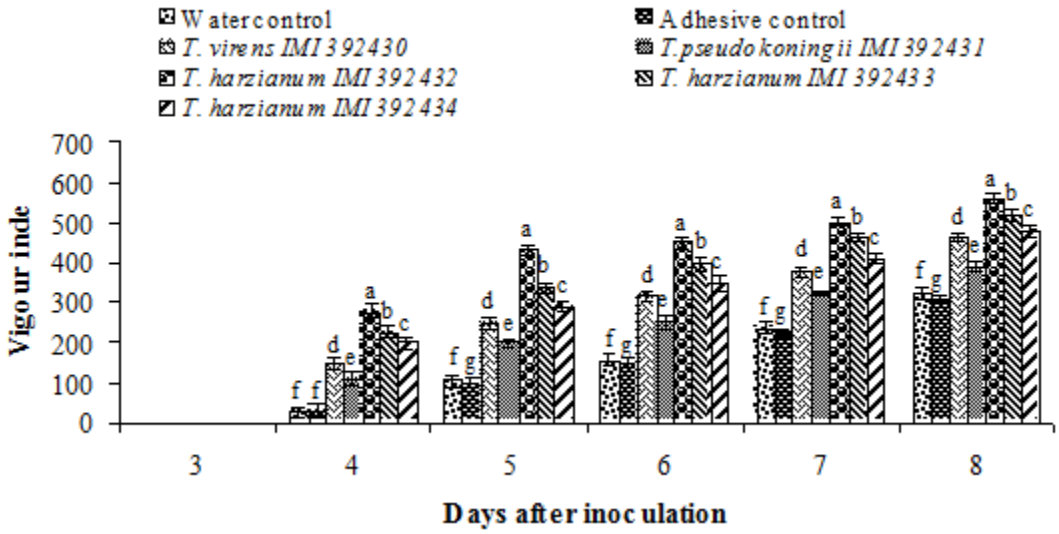

Fig. 4. Effect of seed treatment with five *Trichoderma* strains on vigour index of chili seeds in *in vitro* condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

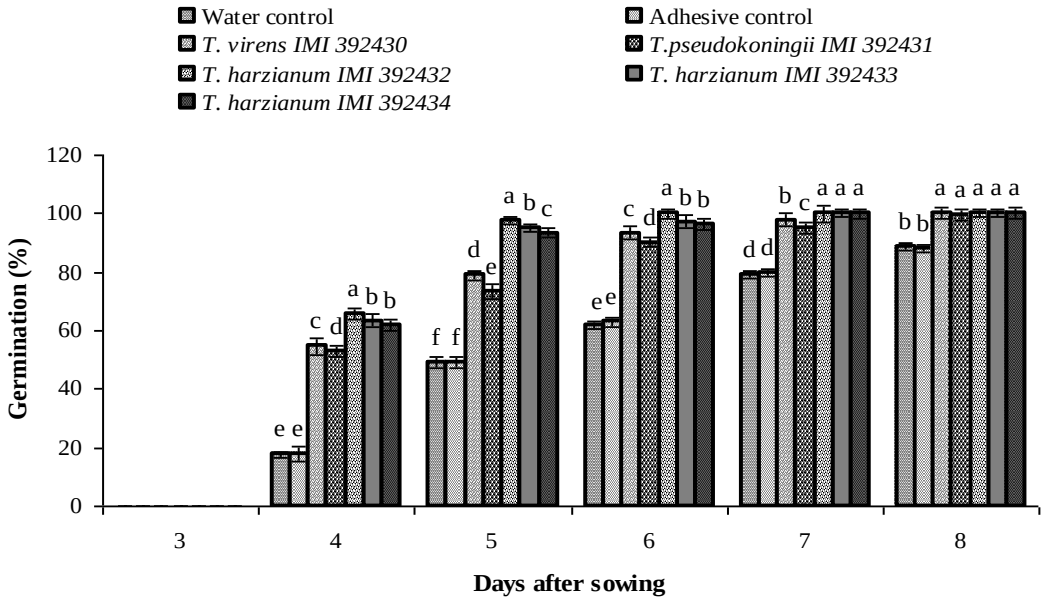

Fig. 5. Effect of seed treatment with culture filtrates of five *Trichoderma* strains on the germination percentage of chili seeds in field condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

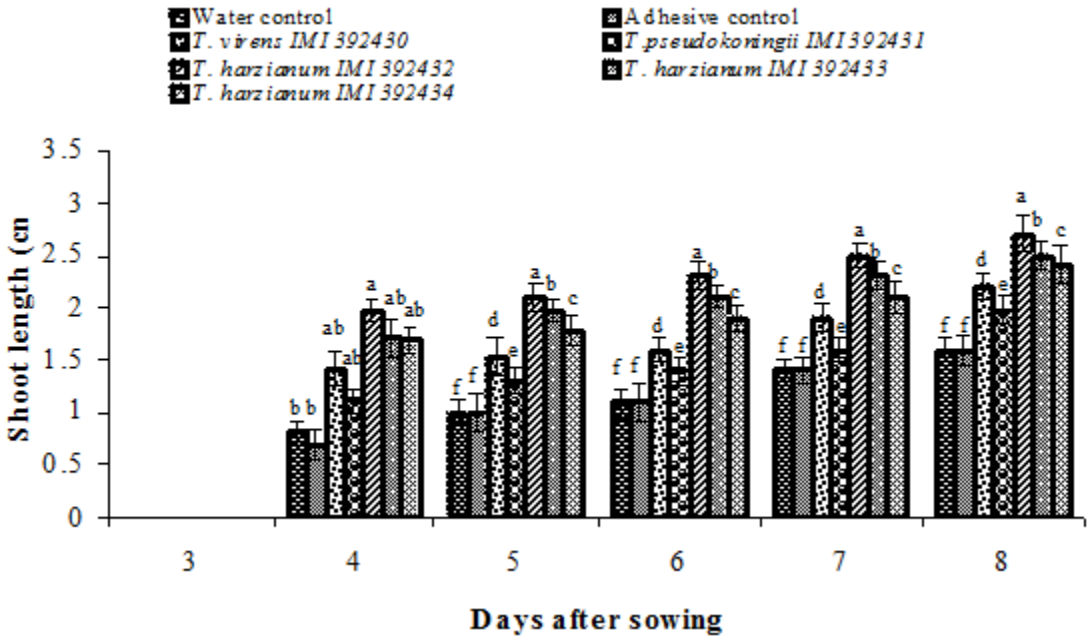

Fig. 6. Effect of seed treatment with five *Trichoderma* strains on the shoot length of chili seeds in field condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

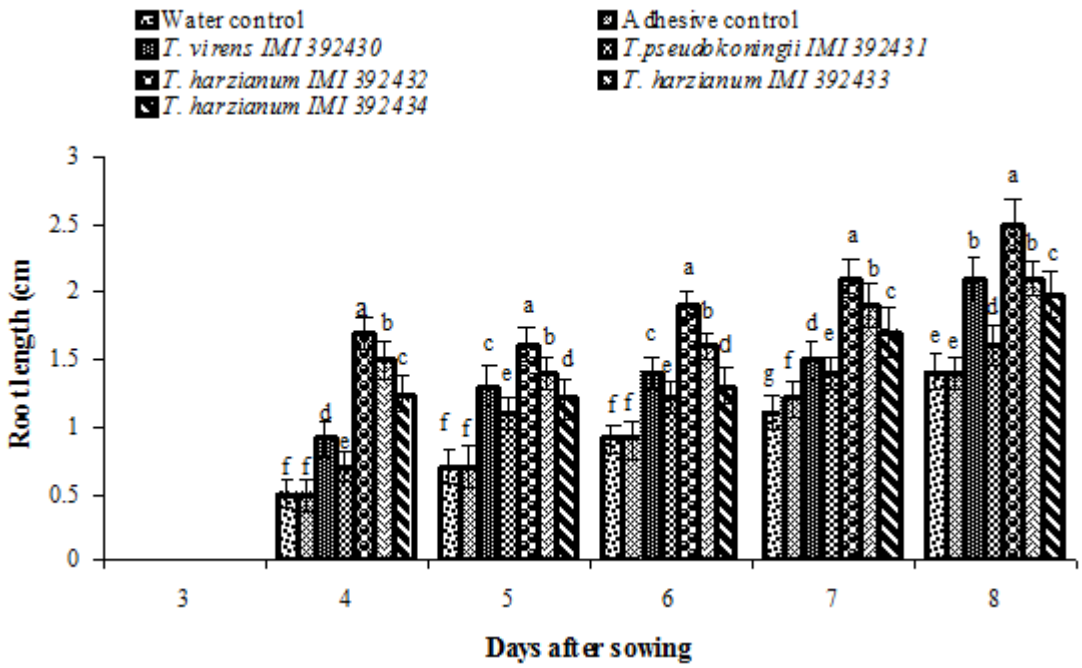

Fig. 7. Effect of seed treatment with five *Trichoderma* strains on root length of chili seeds in field condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

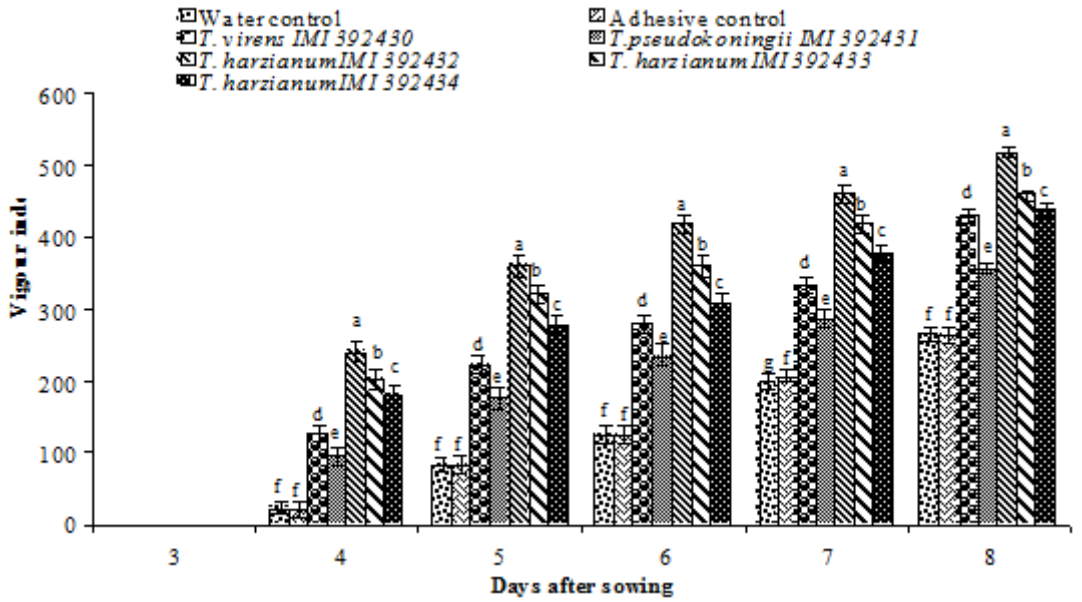

Fig. 8. Effect of seed treatment with five *Trichoderma* strains on vigour index of chili seeds in field condition. Vertical bars show standard error of means of four replicates. Bar marked by the same letters are not significantly different (P<0.05) by DMRT analysis.

Conclusion

The present study concludes that culture filtrates of *Trichoderma* strains 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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