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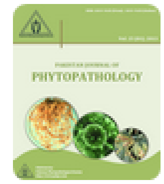

EFFECT OF CULTURE FILTRATES OF *TRICHODERMA* SPP. ON SEED GERMINATION AND SEEDLING GROWTH IN CHICKPEA – AN *IN-VITRO* STUDY

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A B S T R A C T

Trichoderma species are known to produce a number of metabolites that play important role in plant growth, survival and also to enhance defence mechanism of their hosts against certain pathogens. This study was conducted to investigate the effect of *Trichoderma* spp. on the growth activity in chickpea seedlings. Germination percentage of chickpea seeds was enhanced by some *Trichoderma* species culture extracts, the most active stimulators of seed germination were *T. harzianum, T. viride* and *T. koningii* extracts. Results revealed that the maximum seedling root length was obtained by *T. harzianum* followed by *T. viride* and *T. koningii*. However, some species produced extracts that were detrimental to germination and plant growth. The lowest root growth was attained from *T. reesei* and *T. aureoviride* among other *Trichoderma* filtrate treatments. The greatest seedling shoot length was observed with *T. harzianum* and *T. viride* followed by *T. koningii* and *T. pseudokoningii* while other *Trichoderma* cultural filtrates significantly suppressed the shoot length as compare to control value ($P < 0.05$). Fresh and dry weight and of chickpea seedling was also significantly higher in *Trichoderma* treatments as compared to control ($P < 0.05$). However, in *T. reesei* and *T. aureoviride* treatment, fresh and dry weight of seedling was considerably lower where as *T. harzianum, T. viride* and *T. koningii* had higher values for seedling dry matter. Results revealed that *Trichoderma* species are effective for growth parameters and can be used to further investigations and raise the yield of chickpea and other cereal crops.

Keywords: *Trichoderma,* metabolites, chickpea, seedling growth.

INTRODUCTION

Fungi enhance nutrient uptake and can produce growth-promoting metabolites such as gibberellins and auxins in plants that play an important role in growth and their survival (Hamayun *et al.,* 2009). *Trichoderma* is a fungal genus found in many regions of the world. These fungi are ubiquitous in a wide variety of environments, having been found in soil, forests, wood, and paper, among other locations (Akladious *et al.,* 2012). These fungi grow in symbiotic relationships with plants and they promote abundant root growth. *Trichoderma* species have been widely studied for their capacity to enhance plant growth, producing antibiotics, parasitizing other fungi and competing with deleterious plant pathogens so that they are used as biofertilisers and biological agent (Adams *et al.,* 2007). Recently, several attempts have been undertaken to apply *Trichoderma* spp. as bio stimulants of seedling establishment, and to provide enhancement of plant growth and elicit plant defense (Shanmugaiah *et al.,* 2009). Until recently, these traits were considered to be the basis for an indication of how *Trichoderma* can exert beneficial effects on plant growth and development. However, it is becoming increasingly clear that certain strains also have substantial direct influence on plant development and crop productivity (Akladious *et al.,* 2012). Moreover, *Trichoderma*, *Aspergillus* and *Penicillium* species have been reported to produce gibberellin which is a growth regulating hormone in higher plants (Hamayun *et al.,* 2009). Furthermore, all strains of *Trichoderma* species are reported to solubilise metaphosphates and utilize it as phosphorus sources (Phuwiwat and Soytong, 2001). However, inadequate studies are available on chickpea growth stimulants produced by *Trichoderma* species. Therefore, this study aims to find if *Trichoderma* spp.

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have additional promoting effects on seedling growth through the analysis of some indicators, such as percentage of germination, seed vigour, germination index, root length, shoot length, fresh weight and dry weight of chickpea.

MATERIALS AND METHODS

Seed selection: Chickpea seeds of local cultivars were used in the experiment. Healthy seeds were obtained from Institute of Agricultural Sciences, University of the Punjab, Lahore.

***Trichoderma* isolate:** *Trichoderma* species (Table 1) were obtained from the First Fungal Culture Bank of the Pakistan (FCBP), IAGS, University of the Punjab, Lahore, Pakistan. These isolates were kept on Malt extract agar (MEA) (20.0 gm malt extract, 20.0 gm agar and 1.0 L of distilled water) for further study.

Table 1. *Trichoderma* species with their FCBP Accession numbers.

Sr. #	Species	Source	FCBP Accession No.
01	*T. harzianum*	Rhizopsheric soil of Mango, Lahore	FCBP, 860
02	*T. hamatum*	Rhizopsheric soil of *Imperata* sp., Lahore	FCBP, 1126
03	*T. viride*	Rhizopsheric soil of Citrus, Lahore	FCBP, 1123
04	*T. pseudokoningii*	Tannery effluents, Kasur	FCBP, 489
05	*T. koningii*	Rhizosphere of sponge luffa, Changa Manga	FCBP, 1019
06	*T. aureoviride*	Oat seeds, Sargodha	FCBP, 691
07	*T. reesei*	Rhizopsheric soil of *Eucalyptus citriodora,* Lahore	FCBP, 364

Trichoderma* extracts*: For crude fungal extracts preparation, a disc of 1 cm from a 7-day culture of each *Trichoderma* sp. on MEA agar was inoculated into Erlenmeyer flasks (250 cc) containing 100 ml of Malt extract (ME) broth. Culture broth flasks were incubated at 24±2ºC for 15 days. Fungal filtrate was collected by filtration through Whatman filter paper No. 4. Filtrate was stored at 4ºC for further use.

***In vitro*, germination and early seedling growth:** Chickpea seeds were treated with fungal extract by soaking seeds in extract for 30 minutes. For control, seeds were immersed in distilled water. Ten seeds from each treatment were placed on Whatman No.1 filter paper moistened with 3 ml of distilled sterile water in 90-mm-diameter Petri plates. Experimental plates were kept under normal light at 25°C and seed germination progress was recorded daily up to 7 days. The total germination percentage (GP) of germinated seeds was recorded after 7 days of planting (ISTA, 1993; 1999). At harvesting, the seedling height, root length, fresh and dry weights of the root and shoots, germination percentage (GP), germination index (GI), seedling vigour index, were measured.

Germination percentage (GP) was calculated using the following formulas;

$$Germination\ Percentage\ (GP) = \frac{No.of\ germinated\ seeds}{No.of\ total\ seeds} \times 100$$

Seed germination index (GI) was calculated according to Association of Official Seed Analysts (AOSA, 1983) by following formula;

$$GI = \frac{No.of\ germinated\ seeds}{Days\ of\ first\ count}$$

However, Seedling Vigor Index was calculated according to Rahman *et al.* (2012) equation;

Seedling Vigor Index (SVI) = [Mean of root length (cm) + Mean of shoot length (cm) × Percentage of seed germination.

Data Analysis: The average mean of growth parameters from three lab experiments (each experiment was carried out in three replicates) were subjected to analysis of variance and treatment means were computed by Duncan's multiple range test (DMRT) at $P = 0.05$.

RESULTS AND DISCUSSIONS

In present study, seven different extracts of *Trichoderma* spp. were screened and results reveal that some of the *Trichoderma* treatments highly significantly increased the germination of chickpea plants as compared to the control. A significant difference between control and *Trichoderma* extract treatments has been recorded (Table 2).

T. aureoviride and *T. reesei* extract reduced germination in chickpea down to 20%. The lowest germination index of chickpea seed was attained with *T. aureoviride* (1.00), *T. reesei* (1.00), and *T. hamatum* (2.00). It was observed that fungal extract treated seed showed earlier germination after incubation as compared with control seed. It is possible that early emergence of the treated chickpea seed is due to the effect of *Trichoderma* filtrate extract on pre-germination metabolic activities of seed that activate radicle protrusion.

Table 2. Germination percentage, index and vigour of chickpea seeds.

Sr. No.	*Trichoderma* spp.	Germination % age	Germination Index	Seed Vigour
01	Control	50.0	3.0	706
02	*T. harzianum*	90.0	9.0	1657.8
03	*T. hamatum*	40.0	2.0	612
04	*T. viride*	70.0	7.0	1364.3
05	*T. pseudokoningii*	50.0	3.0	734
06	*T. koningii*	70.0	7.0	1364.3
07	*T. aureoviride*	20.0	1.0	457
08	*T. reesei*	20.0	1.0	478

These positive effects supports the stimulatory effects of *Trichoderma* extract on the early stages of germination process, possibly by enhancing the cell division in germinating seeds. Statistical analyses of the data demonstrates a significant difference in root and shoot growth of chickpea seedlings both in *Trichoderma* extract treated seeds and control treatment. Seedling growth promotion by some of the tested *Trichoderma* was significantly higher than the control ($P < 0.05$). The highest seedling shoot length was observed with *T. harzianum* and *T. viride* followed by *T. koningii, T. pseudokoningii* and *T. hamatum* (Figure. 1).

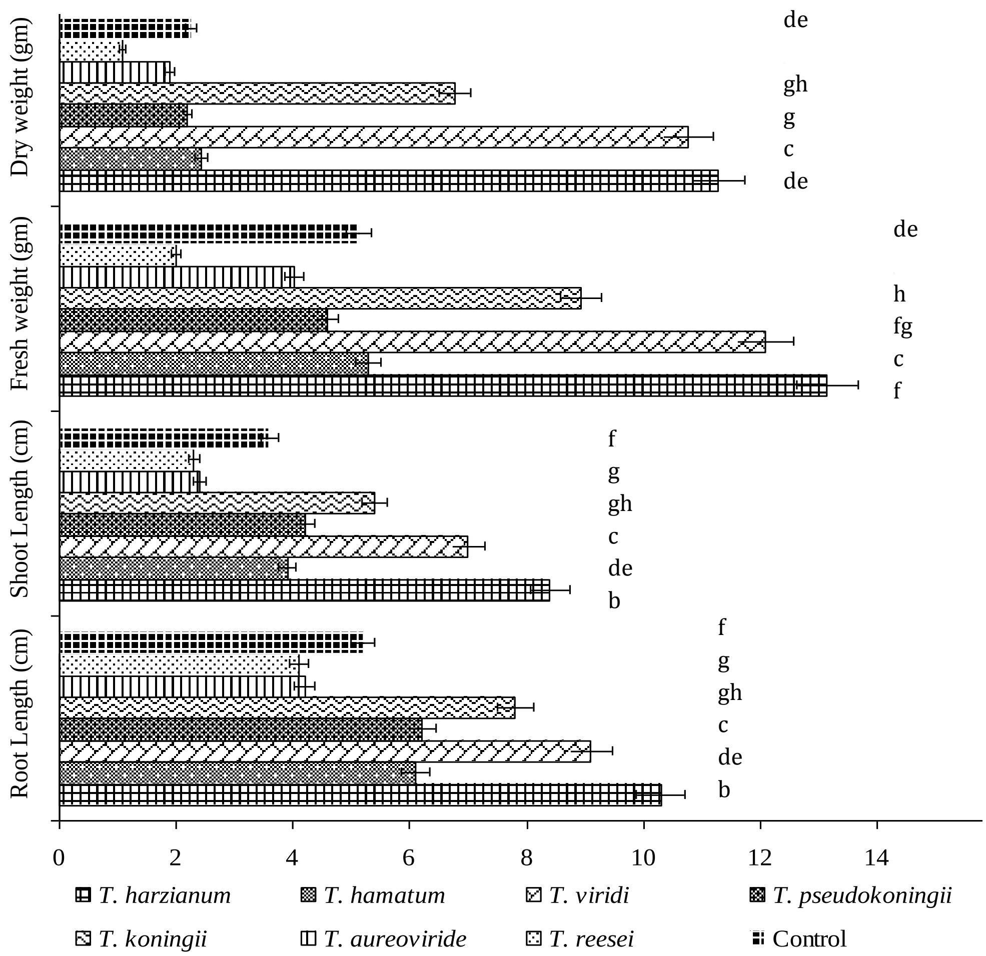

Figure 1. Effect of *Trichoderma* spp. culture filtrates on different parameter [dry weight (gm), fresh weight (gm), shoot length (cm) and root length (cm)] of chickpea seedlings. Bars topped by different letters are significantly different at $P < 0.05$.

Other *Trichoderma* cultural filtrates significantly suppressed the shoot length as compare to control value ($P < 0.05$). Results revealed that the highest seedling root length was attained by *T. harzianum* followed by *T. viridi* and *T. koningii* while, the lowest root growth was attained from *T. aureoviride* and *T. reesei* among other *Trichoderma* filtrate treatments. Current findings showed that *Trichoderma* spp., extract have root length promoting effect and agreed with growth promoting potential of fungal culture filtrate (Rahman *et al.,* 2012). Fresh and dry weight and of chickpea seedling was also significantly higher in *Trichoderma* treatments as compared to control ($P < 0.05$). Although, in *T. reesei* and *T. aureoviride* treatments, fresh and dry weight of seedling was much lower than to the control (Figure. 1). Maximum fresh weight of seedling was recorded with *T. harzianum*, *T. viride* and *T. koningii* treatments that were followed by *T. hamatum* which was significantly higher than the control ($P < 0.05$). However, *T. reesei*, *T. pseudokoningii* and *T. aureoviride* were least effective to stimulate fresh weight than the control. However, results also showed that seedling inoculated with *T. harzianum*, *T. viride* and *T. koningii* had higher values for dry matter as compare to other *Trichoderma* species (Figure. 1).

Present study indicated that different species of *Trichoderma* extract treatments have affected seed growth parameters with a significant difference between control and treated seeds. Mushtaq and Upadhyay, (2011) is also agreed and support that *T. harzianum* used as alternative to the chemicals to suppress the wilt pathogen and raise the yield of tomato. Chlorophyll content increased when seed was coated with *T. harzianum* (Rasool *et al.,* 2011) also some species of *Trichoderma* have the ability to promote plant growth as well as improve plant defence level against biotic and/or abiotic stress (Mastouri *et al.,* 2010). Our results show that the increased growth response of plants caused by application of some species of *Trichoderma* extracts was more effective rather than other treatment, these results indicate that the method of *Trichoderma* introduction is also effective in the success of *Trichoderma* in seedling growth improvement (Akladious *et al.,* 2012). Improved plant growth might be due to increased solubility of insoluble plant nutrients by *Trichoderma* species (Kaya *et al.,* 2009). Hexon *et al.* (2009) showed that *Trichoderma spp*. produced indole-3-acetic acid (IAA) that promoted lateral root formation in *Arabidopsis thaliana.* The root system is important for plant fitness because it provides anchorage, contributes to water use efficiency and facilitates the acquisition of mineral nutrients from the soil (Lopez *et al.,* 2005). Increased root size resulted into increased shoot size which translates into increased shoot biomass production indicating a beneficial effect of inoculation on plant growth and development. The enhanced seedling growth by *Trichoderma* (e.g. *T. harzianum*) might be due to production of secondary metabolites which may act as an auxin like compound; these materials may lead to the development of the root system and an exploration of a large volume of soil (Vinale *et al.,* 2008). Many studies have demonstrated that application of *Trichoderma* increased grain protein content in Chickpea (Alfano *et al.,* 2007). Auxins are important plant regulators involved in many growth and behavioral processes, including those activated by *Trichoderma* spp. (Contreras *et al.,* 2009). Hexon *et al.* (2009) showed that *Trichoderma* spp. produced indole-3-acetic acid (IAA) that promotes lateral root formation. The enhanced plant growth by *Trichoderma* spp. might be due to the production of secondary metabolites which may act as an auxin like compound (Vinale *et al.,* 2008). Seed germination of cucumber increased by application of *Trichoderma,* which may be due to hormonal secretion like gibberellins, auxins (Akhtar *et al.,* 2007). Based on earlier report (Jyotsna *et al.,* 2008) and results presented here we conclude that plant growth may be improved by inoculation with *Trichoderma* spp. which leads to early emergence and also increased vigor of plants. Hence, our findings demonstrate that growth promoting properties of the *Trichoderma* strain improve the efficacy for commercial application.

CONCLUSIONS

The potential of using *Trichoderma* isolates as enhancers of seed germination and plant growth and development could have important economic implications such as shortening the plant growth period and time, as well as improving plant vigor to overcome biotic and/or abiotic stresses, resulting in increase plant productivity and yields. On the basis of results, it is evident that some *Trichoderma* species are effective for growth promotion in chickpea. Further investigation can be carried out to determine the potential of *Trichoderma* spp., on the growth promotion of other crops. Furthermore, the soil microbial communities are suitable for plant growth can also be studied.

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