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Influence of *Trichoderma* species on seed germination in okra

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Abstract

Three *Trichoderma* species were evaluated for their potential regulatory effect. Okra [*Abelmoschus esculentus* (L) Moench] seeds were coated with spore suspension of each test species of *Thichoderma* supplemented with 2% of starch (w/v) as an adhesive. For each treatment, ten seeds were placed in each Petri plate and incubated at 25 °C under dark. Germination of seeds was recorded daily for 10 days. Among the three species, *T. harzianum* was found to be highly effective to enhance the germination percentage in okra seeds. However the species *T. viridi* and *T. koningii* were also significantly effective as compared to control. Okra seeds also gave the highest germination index values with *T. harzianum* which confined to better germination. Seed treatment with *T. harziamun* can be useful to enhance the germination percentage of okra seeds as well as reduce lose due to delayed germination. Further investigations however are required to study *in vivo* effect of *Trichoderma* spp. on germination as well as morphological and physiological characteristics in okra plant and fruit production.
Key words: Okra seeds, seed treatment, *Trichoderma* spp.

Introduction

Trichoderma spp. are beginning employed wiedly in plant agriculture, both for disease control and yield increases (Harman, 2006), even under axenic conditions (Lo *et al.,* 2000; Yedidia *et al.,* 2001). *Trichoderma* spp have evolved multiple mechanisms that result in improvements in plant resistance to disease and plant growth and productivity (Harman *et al.,* 2004; Vinale *et al.,* 2008). Possible explanations of this phenomenon include: control of minor population of pathogens leading to stronger root growth and nutrient uptake (Harman, 2000; Yedidia *et al.,* 2001), secretion of plant growth regulatory factors such as phytohormones (Celar and Valic, 2005; Muthukumar *et al.,* 2005) and release of soil nutrients and minerals by increased saprophytic activity of *Trichoderma* in the soil (Ousley *et al.,* 1994). Moreover, recent studies have indicated that these fungi also induce localized or systemic resistance systems in plants (Yedidia *et al.,* 1999; Howell, 2003; Hanson, 2004). Thus, the variety of effects indicates that these beneficial fungi have multiple modes of action.

Okra (*Abelmoschus esculentus* L.) belongs to family Malvaceae. It is one of the prominent summer vegetable crops grown in Pakistan. Although, okra is an important vegetable crop, but its yield is lower as compared to advanced countries (Usman *et al.,* 2005). Delayed and erratic germination of okra seeds is one of the reasons of low yield of okra. Water imbibitions is 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 (Hydecker, 1977). Delayed and erratic germination creates problems with fertilizer utilization, post emergence weed control, and uniform harvesting (Standifer *et al.,* 1989). The hard seed coat of okra is also a major physiological constraint to uniform stand establishment and performance (Marsh, 1993; Standifer *et al.,* 1989). Therefore, this study was design to find out the effect of different *Trichoderma* species on the germination percentage rate of okra seeds.

Materials and Methods

Spore suspension preparation of *Trichoderma* spp.

Cultures of the *Trichoderma* species i.e. *T. harzianum*, *T. koningii* and *T. viride* were obtained from First Fungal Culture Bank of Pakistan. The test fungi were grown on 2% Maltose Extract Agar (MEA) medium in Petri plats at 28 ^{o}C for 10

days. Spore suspension of each test species was prepared by flooding the plates with sterile distilled water and gently scraping the surface with a sterile spatula. Concentration of spore suspension was adjusted to 10^8 conidia / ml by use of a hemacytometer under a light microscope.

Seed selection and treatment

The okra seeds were one year old and had been stored at 5 °C. Standard germination of the seeds was 80%. Seeds with no cracks or other visible deformations were selected and surface sterilized for 15 minutes with 1% sodium hypochlorite solution. Seeds were then rinsed three times with sterile water and air dried.

A seed coating was prepared from spore suspension supplemented with 2% of starch (w/v) as an adhesive. Dry okra seeds were dipped in seed coating suspension for each *Trichoderma* spp. for 1-2 minutes. For untreated control seeds were dipped in 2% starch suspension and for water control seeds were dipped in water. Seeds were air dry on metallic mash and were placed in Petri plates lines with two layers of Whatman filter paper soaked in sterile distil water. In each Petri plate, ten seeds were placed. Plates were incubated at 25 °C under dark. Germination of coated seeds was compared with untreated control and to a water control Germination of seeds was recorded daily for 10 days. Data were analysed by Duncan's Multiple Range Test. Germination percentage and germination index of okra seeds were calculated as:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds} \times 100}{\text{Total number of seeds}}$$

$$\text{Germination Index} = \frac{\text{Number of germinated seed (first count)}}{\text{Days to first count}} + \frac{\text{Number of germinated seed (2}^{\text{nd}}\text{ count)}}{\text{Days to 2}^{\text{nd}}\text{ count}} + \frac{\text{Number of germinated seed (final count)}}{\text{Days to final count}}$$

Results and Discussion

The effect of three species of *Trichoderma* on germination of okra seeds is shown in Fig 1. Statistical analysis of data showed significant differences in treatments at $p \leq 0.05$ level. Results showed that all the three test *Trichoderma* spp. was found effective to enhance the germination percentage. However among the three species, *T. harzianum* exhibited significant enhancement of germination percentage in okra seeds. However the *T. viridi* and *T. koningii* effectively regulated seed germination as compare to control. Seeds treated with *T. harzianum*, *T. viridi* and *T. koningii* also showed high percentage of germination than control Fig. 1. In controls, both untreated control and water soaked control showed no significant difference was evidenced in germination percentage. Okra seed germination percentage was highly increased by *T. harzianum*. The influence growth promotion by *Trichoderma* is not species specific. Okra seed germination index (GI) also clearly differentiated the potential of different *Trichoderma* spp. (Fig. 2). The results related to germination index showed similar differences as in germination percentages. GI was significantly affected by *Trichoderma* speiecs Fig. 2. Seed treatment with *Trichoderma* spesiecs increased germination index compared to control. Okra seeds gave the highest GI values with *T. harzianum*. The lowest germination index was recorded in control. Seed showed better germination in the presences of *Trichoderma* species. The higher GI results show the higher seed quality and better performance (Wang *et al.,* 2004).

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, three different *Trichoderma* spp gave early germination as well as high 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 (Rojo *et al.,* 2007). Methanol extract of *T. harzianum* and *T. viridi* significantly improved various growth parameters of okra (Prasad and Anes, 2008). Other investigators have also reported that seeds pretreated with *Trichoderma viride, Trichoderma harzianum* and *Trichoderma 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 okra seeds which can be useful to enhance the germination percentage of okra seeds besides reducing loses due to delayed germination. Further investigations are required to study *in vivo,* effects of these fungi on seed germination as well as the morphological and physiological characteristics in okra.

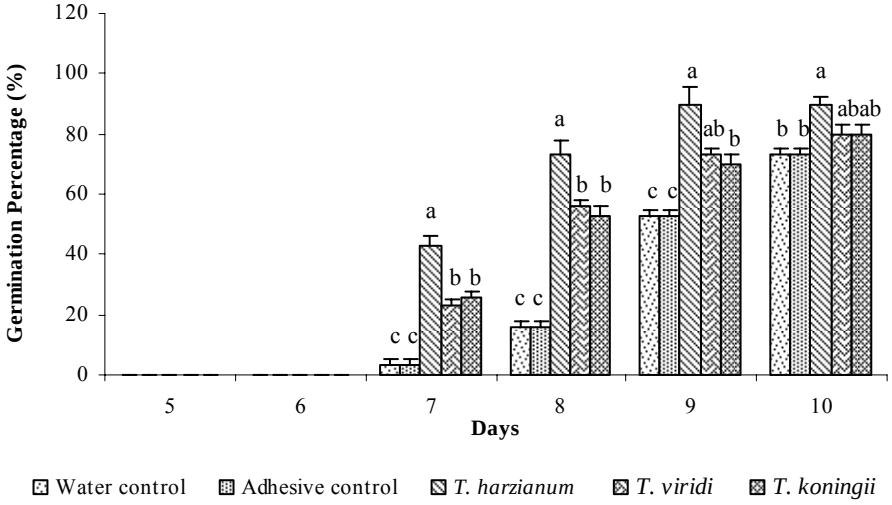

Fig 1. Effect of seed treatment with *Trichoderma* spp on the germination percentage of okra seeds. Vertical bars show standard error of means of three replicales. Values with different letters in a column shows siginficant difference as determin by Duncan's Multiple Range Test ($P \leq 0.05$).

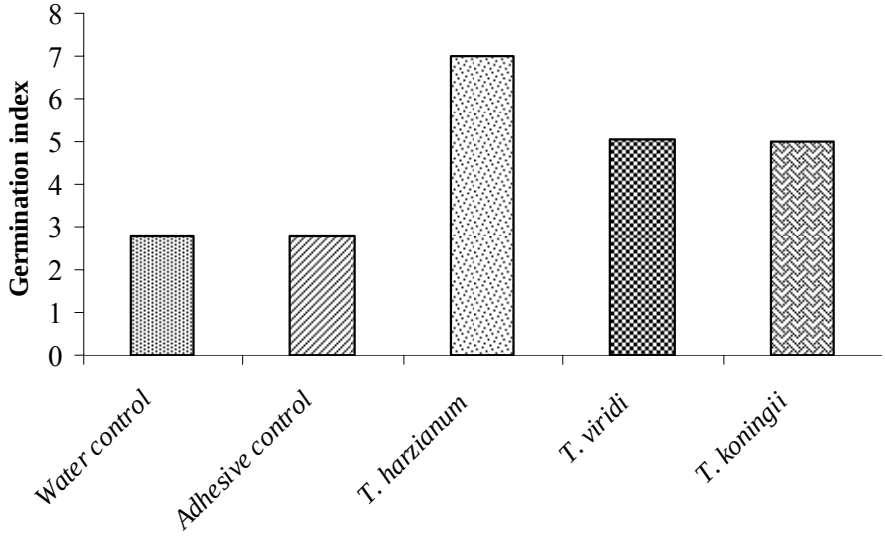

Fig 2. Germination index of okra seeds.

References

Bharath BG, Lokesh S, Prakash HS, Shetty HS, 2006. Evaluation of different plant protectants against seed mycoflora of watermelon (*Citrullus lanatus*). *Res. J. Bot.,* **16**: 1-5.

Celar F, Valic N, 2005. Effects of *Trichoderma* spp. and *Gliocladium roseum* culture filtrates on seed germination of vegetables and maize. *J. Plant Dis.,* **112**: 343-350.

Dubey SC, Suresha M, Singha B, 2007. Evaluation of *Trichoderma* species against Fusarium oxysporum f. sp. ciceris for integrated management of chickpea wilt. *Bioogical Control*, **40**: 118-127.

Hanson LD, 2000. Reduction of *Verticillium* wilt symptoms in cotton following seed treatment with *Trichoderma virens. J. Cotton Sci.,* **4**: 224-231.

Harman GE, Petzoldt R, Comis A, Chen J, 2004. Interactions between Trichoderma *harzianum* strain t22 and maize inbred line mo17 and effects of these interactions on diseases caused by Pythium ultimum and *Colletotrichum graminicola. J. Phytopathol.,* **94**: 147-153.

Harman GE, 2000. Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Dis.*, **84**: 377-393.

Harman GE, 2006. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology,* **96**: 190-194.

Heydecker W, 1977. Stress and seed germination: and agronomic view. In the physiology and biochemistry of seed dormancy and germination, A. A Khan, ed. Amsterdam: North Holland Publishing Company.

Howell CR, 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. *Plant Dis.,* **87**: 4-10.

Lo CT, Liao FT, Deng TC, 2000. Induction of systemic resistance of cucumber to cucumber green mosaic virus by the root colonizing *Trichoderma* spp. (Abstr.) *Phytopathology,* **90:** S47.

Manju S, Mall TP, 2008. Efficacy of *Trichoderma* species on *Phytophthora dresceleri* f. sp. *cajani* of Pigeon pea. *Ann. Plant Prot. Sci.,* **16**: 162-164.

Mishra DS, Sinha AP, 2000. Plant growth-promoting activity of some fungal and bacterial agents on rice seed germination and seedling growth. *Tropical Agric.,* **77**: 188-191.

Muthukumar A, Karthikeyan G, Prabakar K, 2005. Biological control of tuber rot (*Fusarium oxysporum*) tube rose (*Polianthes tuberosa* L.). *Madras Agric. J.,* **92**: 742-744.

Ousley MA, Lynch JM, Whipps JM, 1994. Potential of *Trichoderma* spp. as consistent plant growth stimulators. *Biol. Fertil. Soils,* **17**: 85-90.

Oyarbide F, Osterrieth ML, Cabello M, 2001. *Trichoderma koningii* as a biomineralizing fungous agent of calcium oxalate crystals in typical Argiudolls of the Los Padres Lake natural reserve (Buenos Aires, Argentina). *Microbiol. Res.,* **156**: 113–119.

Prasad D, Anes KM, 2008. Effect of metabolites of *Trichoderma harzianum* and *T. viride* on plant growth and meloidogyne incognita on okra. *Ann. Plant Prot. Sci*., **16**: 461-465.

Rabeendran N, Moot DJ, Jones EE, Stewart A, 2000. Inconsistent growth promotion of cabbage and lettuce from *Trichoderma* isolates. *New Zealand Plant Prot.,* **53**: 143-146.

Rojoa FG, Reynosoa MM, Fereza M, Chulze SN, Torres AM, 2007. Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. *Crop Prot.,* **26**: 549-555.

Standifer LC, Wilson PW, Drummond A, 1989. The effects of seed moisture content on hardseededness and germination in four cultivars of okra [*Abelmoschus sculentus* (L) Moench]. *Plant Varieties Seeds,* **2**: 149-154.

Usman K, Ahmad E, Khan MU, Ahmad A, Imdad A, Iqbal J, 2005. Integrated weed management in okra. *Pak. J. Weed Sci. Res*., **11**: 55-60.

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL and Lorito M, 2008. *Trichoderma*–plant–pathogen interactions. *Soil Biol. Biochem.*, **40**: 1-10.

WangYR, Yu L, Nan ZB, Liu YL, 2004. Vigor tests used to rank seed lot quality and predict field emergence in four forage species. *Crop Sci*., **44**: 535-541.

Yedidia I, Benhamou N, Chet I, 1999. Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. *Appl. Environ. Microbiol.,* **65**: 1061-1070.

Yedidia I, Srivastva AK, Kapulnik Y, Chet I, 2001. Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. *Plant Soil,* **235**: 235-242.

Zheng Z, Shetty K, 2000. Enhancement of pea (*Pisum sativum*) seedling vigour and associated phenolic content by extracts of apple pomace fermented with *Trichoderma* spp. *Process Biochem.,* **36**: 79-84.