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Research Article

Trichoderma harzianum isolates of Indo-Gangetic plains as antagonists and growth promotion agent for okra

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ABSTRACT: Seven *Trichoderma harzianum* isolates from Indo-gangetic plains of Kanpur area were screened for their biocontrol potential and ability to promote germination and growth in *Abelmoschus esculentus* seeds in *invitro* conditions. Four superior isolates (ThS, ThP, ThV, ThK) were multiplied on rice husk bran (RHB), shade dried and powdered. Okra seeds were treated (@ 6g/kg) with *T. harzianum* inoculum and sown in the field. The treated seeds evinced early seedling emergence and bud initiation, increase upto 31-48% in plant height, 27-42% in fruit length, 86-110% in fruit fresh weight and 52-87% in yield was noticed. Treatment with the isolate ThS was most superior. All the isolates used for seed treatment have more or less equal biocontrol potential but there was a great variation in their growth promotion ability both lab and field.The study indicates that biocontrol and growth promotion ability of *T. harzianum* isolates are independent of each other.

KEY WORDS: *Trichoderma harzianum*, *Abelmoschus esculentus*, biocontrol, growth promotion, seed treatment

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INTRODUCTION

Trichoderma species are one of the most extensively studied biocontrol fungi used for the management of various plant pathogens. Several mechanisms such as mycoparasitism, antibiosis, nutrient competition starvation and induction of systemic resistance etc. are employed by *Trichoderma* during biocontrol (Dennis and Webster, 1971; Upadhyay and Mukhopadhyay, 1986; Chet, 1987; Howell, 2003).

Trichoderma species are also reported to improve the vegetative growth and yield of crop plants (Singh and Kumar, 2011). Various formulations of *Trichoderma* have been developed and are being applied by farmers in many crop production systems. But, many of these biocontrol agents frequently fail to perform or under-perform in the field conditions. The reasons behind this non-performance may be biotic or abiotic factors such as competition with local microbes, low colony forming units, local environment unsuitable for growth and proliferation, poor nutritional status of soil,etc. To overcome the above problems, formulations containing local isolates of biocontrol agents may be more useful as they are well adjusted to the environmental conditions.

The present study was undertaken to isolate local strains of *Trichoderma* from Indo-Gangetic plains (IGP) and to identify their growth promotion ability. *Abelmoschus esculaentus* (okra) is an important vegetable crop grown throughout the globe and is also widely cultivated in IGP of Kanpur area, therefore it was selected for the study.

MATERIALS AND METHODS

Isolation of *Trichoderma* from Indo-Gangetic plains (IGP)

Fifteen soil samples were collected from fields of Kanpur and its adjoining areas located near the banks of Ganga River. Samples were dried under laminar air flow, crushed-to make powder and serial dilution technique was used for isolation of *Trichoderma* (Elad *et al.* 1981). One ml of serially diluted soil solution wasspread on the *Trichoderma* Selective Medium (TSM) in Petri plates and incubated at 25±2°C. The colonies observed were sub-cultured on Potato dextrose agar (PDA), purifiedby Single spore isolation technique (Choi *et al*., 1999) and identified as *Trichoderma harzianum* by morphological means.

Biocontrol Potential of *Trichoderma* isolates

All the 7 *T. harzianum* isolates were evaluated for their biocontrol potential by Dual culture technique (Dennis and Webster, 1971). *T. harzianum* isolates and *Fusarium oxysporum* (Fo) a wilt pathogen were grown separately on PDA Petri plates. After 7 days, 5 mm bits of cultures were cut

and each *T. harzianum* isolate was placed opposite to the pathogen on 90mm Petri plates. The experiments were repeated twice and % inhibition was calculated using the formula:C – T/ C x 100, where C = Growth of Fo in control plate, T = Growth of Fo in dual culture plate.

Mass Production of *Trichoderma*

Trichoderma harzianum isolates were multiplied on rice husk bran medium (RHB), which contained rice husk and rice bran in a ratio of 3:1. Hundred gram of RHB was filled in autoclavable polybags, moistened,sterilized thrice at 121°C for 15 min, inoculated with 5ml of spore suspension (Cfu - 10^7) of *T. harzianum* isolates and then incubated at 25±2°C. After 15 days of incubation, the mixture was dried in shade, powdered and used as inoculum to treat the okra seeds for trails.

Seed Germination and Growth Promotion under *in vitro* conditions

Effect of *T. harzianum* isolates on seed germination and growth promotion was studied by paper roll towel method (ICRISAT Seed Processing Manual).Water primed okra seeds were treated (@ 6 gm/kg seed) with *T. harzianum* isolates inoculums using 20% aqueous gum Arabic solution and dried under shade for 6 hours. Hundred seeds from each treatment were placed between two blotting papers, moistened with sterile water and kept at 25±2°C. Untreated seeds served as control. The trials were conducted twice and data was recorded for % germination, root and shoot length and seedling vigour index was calculated.

Plant growth promotion under field conditions

Twenty five seeds of okra treated with different isolates of *T. harzianum* were planted in pre-prepared plots at spacing of 45x30cm. Data on first seedling emergence, first bud initiation and flowering, plant height, fruit length, fresh weight of fruit and fruit yield was recorded. Three replicate plots were maintained for each treatment and experiments were conducted twice.

RESULTS AND DISCUSSION

Isolation of Antagonists from soil

A total of 7 *Trichoderma harzianum* (*T. harzianum*) strains were isolated from 15 soil samples of Kanpur IGP area. Around 50 % (7 out of 15) soil samples collected from different locations revealed presence of the biocontrol agent.

Effect of *T. harzianum* on *Fusarium oxysporum*in dual culture

Dual culture data indicates that all the *T. harzianum* isolates have high biocontrol potential against *Fusarium oxysporum*. Mycelial growth inhibition of 60-69% was recorded by *T. harzianum* isolates, maximum with ThP and minimum with ThB. Per cent inhibition caused by ThP and ThS was significantly (P>0.001) higher than all the other isolates. There was no significant (P>0.001) difference between ThA, ThV, ThK and ThI (Fig. 1). All the *T. harzianum* isolates successfully colonized RHB medium and produced high colony forming units (10^8/g).

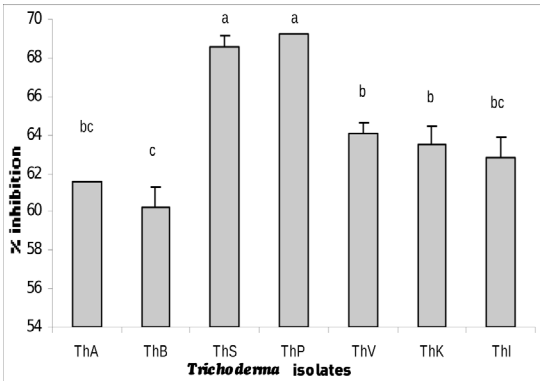

Fig. 1. Per cent inhibition of *Fusarium oxysporum* with 7 isolates of *Trichoderma harzianum*. Data is means of two experiments with 3 replicates with standard error (±). Columns containing same letter do not differ significantly, according to Duncuns test (P>0.001).

Effect of *Trichoderma harzianum* treatment on seed germination in roll towel

Seeds treated with *T. harzianum* isolates showed high germination per cent in roll towel experiments compared to untreated ones. Germination of seeds ranged from 79-94%, lowest with ThB and highest with ThS. Per cent increase in germination with different isolates ranged from 1.7 to 20, and ThS was more effective (Fig. 2). Seeds treated with isolate ThA, ThB and ThI do not differ significantly (P>0.001) with each other.

Effect of *T. harzianum* treatment on seedling length and vigour in roll towel

Variation in okra seedlings length was observed and growth was significantly (P>0.001) increased with ThS (73%) and minimum with ThB (3%)(Table1). There was no significant difference between ThA, ThB, ThI and control with regard to seedling length. ThS, ThP, ThV, ThK were superior among all the 7 isolates and showed increased seed germination, growth promotion and vigour index and therefore were selected for field trials.

Effect of *T. harzianum* on seedling emergence, flowering and plant height in fieldconditions

Seedling emergence was recorded on 3rd day after sowing in the treated okra seeds while as in control plots

Table 1. Effect of *Trichoderma harzianum* treatment on seedling vigour and germination index in okra seeds.

Trichoderma isolate	Seedling ht (cm)*	% Increase	% Germination*	% Increase	Germination Index
ThA	9.90^{c}	5.32	80.17^{c}	2.56	793.68
ThB	9.65^{c}	2.65	79.50^{c}	1.70	767.18
ThS	16.35^{a}	73.94	94.16^{a}	20.47	1539.52
ThP	13.45^{b}	43.08	88.50^{b}	13.22	1190.33
ThV	12.90^{b}	37.23	88.30^{b}	13.01	1139.07
ThK	12.70^{b}	35.10	87.50^{b}	11.94	1111.25
ThI	10.15^{c}	7.97	81.16^{c}	3.84	823.77
Control	9.40^{c}		78.17^{c}		734.80

*Value in column containing same letter do not differ significantly, according to Duncuns test (P>0.001).

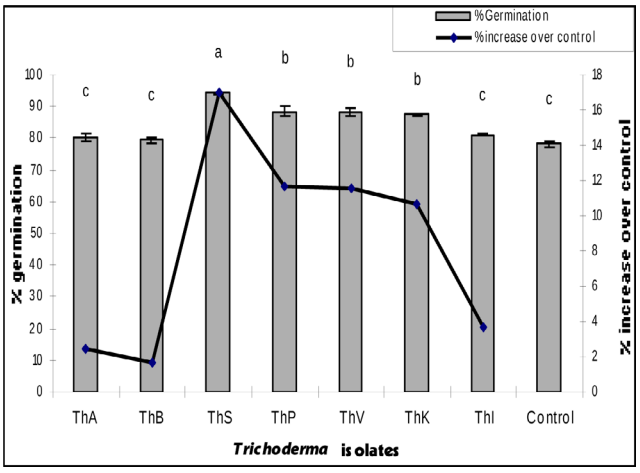

Fig. 2. Effect of *Trichoderma harzianum* treatment on per cent germination in okra seeds using roll towel method. Data represents of two experiments with 3 replicates with standard error (±). Columns containing same letter do not differ significantly, according to Duncuns test (P>0.001).

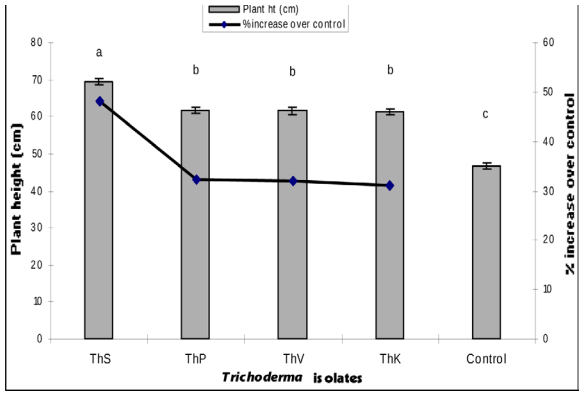

Fig. 3. Effect of *Trichoderma harzianum* treatment on plant height of okra and per cent increase over the control. Data repersent of two experiments with 3 replicates with standard error (±). Columns containing same letter do not differ significantly, according to Duncuns test (P>0.001).

seedling emergence was observed on 5th day. First bud formation was recorded at 30, 35, 36, 36 and 40 days after sowing in the plots treated with ThS, ThP, ThV, ThK and control respectively (Fig. 3). Plant height measured at 60 days shows that all the isolates have significant (P>0.001) growth promotion effect, highest with ThS (69.3 cm). Plant height of okra treated with ThP, ThV and ThK were significant (P>0.001) than control but they do not differ among themselves.

Effect of *T. harzianum* on yield parameters of okra in fieldconditions

Fruit size of okra ranged from 10.7-15.2cm. ThS treated seeds were having significantly (P>0.001) higher fruit length compared to other treatments. There was no significant difference among ThP, ThV and ThK with regard to

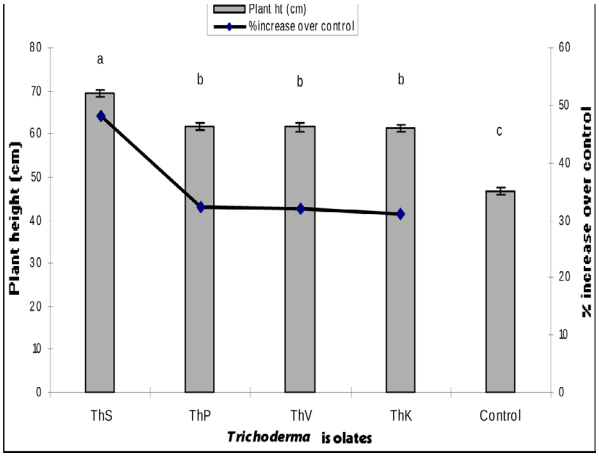

Fig. 4. Effect of *Trichoderma harzianum* treatment on fruit length of okra and per cent increase over the control. Data represent of two experiments with 3 replicates with standard error (±). Columns with same letter do not differ significantly, according to Duncuns test (P>0.001).

fruit length (Fig. 4). Early fruit formation was recorded in all the treatments compared to control. Maximum (2.24kg/plot) fruit production was recorded in the plots treated with ThS, followed by ThV (1.98 kg/plot), ThP (1.97 kg/plot) and ThK (1.83 kg/plot). Plants treated with ThS isolate obtained significantly (P>0.001) higher fruit as compared to others (Table. 5). Ability of *T. harzianum* isolates to promote fruit formation and increase yield is well proved in by the above experiments.

Isolation of 7 *Trichoderma harzianum* (*T. harzianum*) strains from 15 soil samples proves the dominant existence of native biocontrol agents. Presence of local potential biocontrol agent is beneficial to crops as they are well adapted and have advantage to compete with foreign and pathogenic strains.

Dual culture data is indicates that all the 7 *T. harzianum* isolates have great capability to control *Fusarium oxysporum*. Mycelial growth inhibition of *F. oxysporum* by *T. harzianum* isolates ranged from 60-69%, maximum with ThP.Orole and Adejumo (2009) reported radial growth inhibition of *Fusarium* by *Trichoderm a*ranging from 25-75%. Cundom *et al.*(2003) reported inhibition of *R.solani* from 38-59% with 9isolates of *Trichoderma*. ThP and ThS were found more superior to other isolates and this may be due to increased presence of one or more than one mechanism of biocontrol operated by *T. harzianum* isolates in the presence of *F. oxysporum*. Abilities such as secretion of volatile compounds and enzymes, faster growth, utilization of growth medium, etc. of any bio-entity makes its more attractive tool of bio-control. Mass production of bio-agents is an important parameter which should be taken into consideration when they are isolated, screened and selected for application in field conditions. *T. harzianum* isolates multiplied on RHB mediumproduced higher number of viable cell count. The strains which have higher ability to utilize the agriwaste, grow and multiply rapidly and produce high colony forming units. Variations in the potential of *Trichoderma* to produce colony forming units on different substrates have been reported by several workers (Gangadharan and Jeyarajan, 1990; Prakash *et al.* 1999; Sawant and Sawant 1996). Parab *et al.* (2008) reported that rice bran agriwaste was supportive for faster growth and coverage of *T. harzianum* and resulted in high cfu production.

Okra seeds treated with *T. harzianum* germinated faster and also the germination per cent was higher in roll towel experiments. Higher seed germination (79.5-94%) may be due to availability of different favourable conditions of growth such as balanced nutrients, presence of growth hormones or substances, pathogen free environment etc. Isolate ThS was having highest positive effects on the germination, growth and seedling length.Seeds treated with ThS evinced highest seed vigour index, which is an important

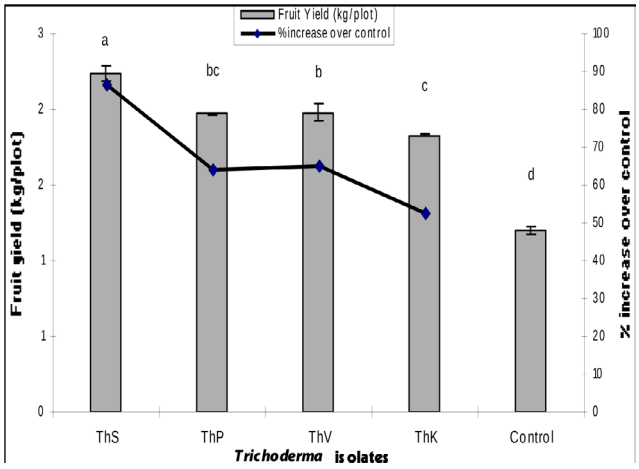

Fig. 5. Effect of *Trichoderma harzianum* treatment on fruit yield in okra and per cent increase over the control. Data is means of two experiments with 3 replicates with standard error (±). Columns containing same letter do not differ significantly, according to Duncuns test (P>0.001).

indicator that it has high ability to promote germination in okra. Islam *et al.* 2011) carried out germination experiments in chilli seeds and found that among five *Trichoderma* strains, *T. harzianum* IMI-3924332 gave the highest germination per cent followed by *T. harzianum* IMI-3924333, *T. harzianum* IMI-3924334, *T. virens* IMI-392430 and *T. pseudokoningii* IMI-392431.

Trichoderma harzianum treatments resulted in faster emergence of okra seedlings, early bud formation and flowering and increased plant height, maximum with ThS. Favourable conditions (abiotic and biotic) are known to have positive effects on different growth parameters of a crop. In the above experiments, all the treatments were provided with same abiotic conditions and only the biotic conditions was modified by inoculating isolates of *T. harzianum.* Similar were the findings of Mishra and Sinha (2000) and Ashraf *et al.* (2005) who observed the growth promoting activity of different microbial isolates on rice seed germination and seedling growth. Hamed *et al.* (2011) reported that *Trichoderma* isolates were capable of increasing the seed germination, growth and germination index of the two varieties of muskmelon.

Fruit length and yield data indicates that different isolates of *T. harzianum* have differential growth promotion ability and ThS was having significantly highest potential. Field trial results were almost similar to the effectiveness recorded under *invitro* conditions. Present study clearly indicates that in addition to biological control phenomenon, *T. harzianum* isolates also have potential to promote the growth and yield of okra. A significant increase in the growth and yield parameters of the soybean treated with *Trichoderma* was also reported by John *et al.* (2010). They

found that there were 66 fruits per plant in the treated ones however, in control only 41 fruits per plant were recorded. Begam *et al.,* (2010) reported decrease in fruit rot and increase in the growth and yield of chilli when they were treated with *Trichoderma* species.

Results clearly indicate that biocontrol potential and growth promotion ability of *T. harzianum* isolates are independent of each other. Local *Trichoderma* isolates have great potential to promote the growth and yield of okra crop

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