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Research Article

Trichoderma-enriched coco-peat for the management of *Phytophthora* and *Fusarium* diseases of chilli and tomato in nurseries

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ABSTRACT: Coconut coir dust, commercially available as coco-peat, is used in raising the seedlings of vegetable crops in tropical countries. Coir-pith and other derivates of coconut husk have been well recognized as substrates for the multiplication of *Trichoderma* spp. and commercial nurseries use coco-peat for raising the seedlings. In the present study, coco-peat enriched with *Trichoderma harzianum* was used for raising tomato and chilli seedlings to test the effect of the same on managing wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* in tomato and damping off and root rot caused by *Phytophthora capsici* in chillies in nurseries. The enrichment with *T. harzianum*, resulted in reduced wilt incidence (5-7.5%) compared to control (38.75%) in tomato with increased plant growth parameters. Though germination was reduced compared to control (without pathogen), there was reduction in *P. capsici* infection in chillies by up to 50% compared to coco-peat without *Trichoderma* enrichment. The use of coco-peat enriched with *T. harzianum* can be adopted by commercial nurseries for better plant growth and reduced incidence of tomato wilt and chilli root rot while raising disease free and healthy seedlings.

KEY WORDS: *Trichoderma* spp., coco-peat, *Fusarium oxysproum* f. sp. *lycopersici*, *Phytophthora capsici*, tomato, chilli, commercial nurseries.

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INTRODUCTION

Coconut coir dust, commercially known as coco-peat, is an easily affordable growth medium for raising seedlings in nurseries, especially for vegetable crops in the tropics. Coco-peat is an agricultural by-product obtained after extraction of fiber from the coconut husk (Abad *et al.*, 2002; Yahaya *et al*., 1999, 2009). Coco-peat has good water-holding capacity, acceptable electrical conductivity and other chemical attributes. It has a pH of 5.2-6.8 which is neutral to slightly acidic. Coco-peat has the ability to store and release nutrients to plants for extended periods of time. It also has great oxygenation properties which is important for healthy root development. The coco-peat is reusable and hence preferred by nursery growers for raising seedlings. It can be combined with any of the normal ingredients and used as a mixer or a stand-alone product. It is available at an affordable price.

Nursery diseases of vegetables are very important as the production of disease free seedlings is a critical requirement for the production of healthy crop in the main field. If seed borne diseases and seedling related diseases are contained at an early stage using bio-intensive practices, the disease free seedlings would be available for the main crop without much need for chemical interventions. Wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* and root rot or wilt by *Phytophthora capsici* in chillies and other *Capsicum* spp. are important in the nurseries. There are many reports on the use of coir-pith as a substrate for the mass production of *Trichoderma* (Saju *et al*. 2002; Jeyarajan, 2006; Usharani *et al.*, 2008). In the present work, the use of sterilized or unsterilized coco-peat for *Trichoderma* enrichment and the effect of using the enriched coco-peat that was used to raise seedlings on the *Fusarium* wilt incidence in tomato and *P. capsici* infection in chili were studied.

MATERIALS AND METHODS

Commercial grade coco-peat available as Bio-peat (SG) compost which is made of coconut coir dust and generally used in horticultural nurseries was used in the study.

Microbial population in coco-peat

To assess the natural microflora in coco-peat, one g of coco-peat was taken in nine ml sterile water and serially diluted. One ml of 4th and 5th dilutions were plated on nutrient agar (NA) and potato dextrose agar (PDA). One ml of 2nd and 3rd dilutions was plated on *Trichoderma*-specific medium and all the plates were incubated at ambient temperature (25±2°C) for three days. After incubation, the colonies were enumerated and recorded.

Production of Trichoderma *inoculum*

Trichoderma harzianum – NBAII-Th10 (MTCC 5584 deposited a IMTECH, Chandigarh, India) isolate maintained at National Bureau of Agriculturally Important Insects (Formerly Project Directorate of Biological Control), Bangalore was used. It was grown on PDA at 25°C for seven days. The spore suspension was prepared by flooding the plate with sterile water and gently scraping with a sterile glass rod. The suspension was collected and its concentration was adjusted to 2 x 10^6 spores ml^{-1} using a haemocytometer. One ml spore suspension was used as inoculum and transferred to 200 ml of sterilized potato dextrose broth (PDB) and incubated in a rotary shaker at 150 rpm for 96 hours at 28°C temperature. After incubation viability in terms of colony forming units (CFUs) was checked.

Substrate preparation, inoculation and incubation

Coco-peat was taken in autoclavable polypropylene bags (20cm x 30cm) at the rate of 100 g per bag and autoclaved at 121°C for 30 min. for two consecutive days. One set of polypropylene bags with coco-peat without sterilization was also maintained for comparison. Ten ml of *T. harzianum* inoculum was transferred to each bag containing 100 g coco-peat under aseptic condition and incubated at ambient temperature (28°C). Viability of *T. harzianum* in terms of CFUs in each bag containing coco-peat was assessed at weekly interval for six weeks.

Production of Fusarium oxysporum *f. sp.* lycopersici *inoculum*

A nine mm mycelial disc of *F. oxysporum* f. sp. *lycopersici* from seven day-old culture was transferred to 100 ml of sterilized potato dextrose broth and incubated in a rotary shaker at 150 rpm for five days at ambient temperature (25± 2°C). After incubation the inoculum was filtered through cheese cloth and the conidial suspension obtained was adjusted to 1x10^5 conidia ml^{-1} using a haemocytometer.

Production of Phytophthora *capsici inoculum*

A nine mm mycelial disc of *P. capsici* was transferred to 50 ml of sterilized half-strength carrot dextrose broth in flat bottles and incubated at ambient (25±2°C) temperature for five days. The broth was filtered through cheese cloth and the sporangial count was adjusted to 4 x 10^5 sporangia ml^{-1} with a haemocytometer. For sporulation the flasks were subjected to cold shock at 4°C for 2 hours and then placed outside under artificial light for 24 hours for zoospore release.

Bioefficacy tests for the management of P. capsici *infection in chilli and* Fusarium *wilt in tomato*

Seeds of 'Byadagi kaddi', a chilli cultivar susceptible to *P. capsici* and seeds of tomato variety *Pusa Ruby* susceptible to *F. oxysporum* f. sp. *lycopersici* were used. Seeds were surface sterilized with sodium hypochlorite solution (0.01%) for 2-3 min and thoroughly washed with sterile distilled water before sowing. Pots of 15 cm diameter were used to raise the plants. The treatments tested were (i) sterilized coco-peat enriched with *Trichoderma* (SCP), (ii) unsterilized coco-peat (USCP) enriched with *Trichoderma*, (iii) and un-autoclaved coco-peat without *Trichoderma* enrichment Each pot was filled with 40 g of un-autoclaved coco-peat + Th-10, autoclaved coco-peat + Th-10 or un-autoclaved coco-peat. One set of pots without Th-10 enrichment was maintained for comparison.

Before sowing, the pots were inoculated with 10 ml of sporangium or conidial suspension of *P. capsici* or *F. oxysporum f. sp. lycopersici* for respective bioassays. Ten seeds were placed in each pot and four replicates were maintained for each treatment. The roots were drenched with 10 ml of conidial suspension of *F. oxysporum f. sp. lycopersici* after two weeks or sporangial suspension of *P. capsici* after three weeks. The plants were maintained for 45 days. The final disease incidence was recorded along with other parameters such as germination percentage, disease incidence, root colonization (by plating 25 root bits on *Trichoderma*-selective medium (Elad *et al.,* 1981) after surface sterilization) and plant growth parameters (root and shoot length). The data were analysed statistically using analysis of variance (ANOVA) using RBD design (Gomez and Gomez, 1984). The values of CFUs were log transformed (base to 10) before analysis.

RESULTS AND DISCUSSION

Microbial population in coco-peat

On NA and PDA different bacterial and fungal colonies were observed respectively when serial dilution plating was done from one g of coco-peat. Bacterial colonies could be detected even at 10^{-5} serial dilution while number of fungal colonies were too numerous to count (<100) at 10^{-4} dilution and at 10^{-5} dilution seven colonies were recorded. Native *Trichoderma* colonies on the commercial coco-peat could not be traced on TSM.

Growth and viability of Trichoderma *on sterilized and un-sterilized coco-peat*

T. derma harzianum (NBAII-Th10) could multiply on both sterilized and un-sterilized coco-peat. The average viability of Th10 immediately after inoculation of the substrate was 1 x 10^6 CFUs g^{-1}. Within one week, the

viability of Th-10 in coco-peat increased to $>10^7$ CFUs g^{-1}. Up to seven weeks the viability did not reduce in both sterilized and un-sterilized coco-peat. The results indicated that in autoclaved coco-peat the viability was higher (7.83 Log CFUs g^{-1}) compared to unsterilized one (6.90 Log CFUs g^{-1}) (Table 1). At any observation time, the viability in autoclaved coco-peat was higher than that of unsterilized coco-peat. The maximum viability was observed when samples were drawn on 14th or 21st day after inoculation indicting that the coco-peat has to be used before 21 days. After 21 days, in autoclaved coco-peat there was no significant change in viability while in unsterilized coco-peat there was a decline in the viability of Th-10.

The increase in the total population density of *T. harzianum* in unsterilized coco-peat suggests that competition by native fungi or bacteria was not a limiting factor in colonization by Th10, though the increase was not on par with that observed in sterilized coco-peat.

Bioefficacy test against Fusarium *wilt in tomato*

Germination of tomato seeds did not differ significantly either in the presence or absence of *F. oxysporum* f. sp. *lycopersici*. Similarly enriching coco-peat with Th-10 either after sterilization or without sterilization did not significantly alter the germination percentage (85-97.5%), though the maximum per cent germination (97.5%) was noticed in sterilized coco-peat that was enriched with Th-10 (Fig. 1A).

However, the per cent wilt incidence in the presence of pathogen significantly differed between sterilized and unsterilized coco-peat. In coco-peat without *Trichoderma* enrichment, there was 38.75% wilt incidence while in coco-peat enriched with Th-10 after sterilization of the substrate it was 7.5% and in unsterilized coco-peat with Th-10 enrichment it was 5% (Fig. 1A).

The average shoot length and root length were 9.5 cm and 2.8 cm, respectively, in *Fusarium* inoculated plants planted in coco-peat without *Trichoderma* enrichment. The shoot length was 14.3 cm and 17.3 cm in plants inoculated with *Fusarium* and planted in sterilized and unsterilized coco-peat respectively. The root length was 7.47 cm and 3.5 cm in plants inoculated with *Fusarium* and planted in sterilized and unsterilized coco-peat respectively (Fig. 2A). In the absence of the pathogen, root length was more in the plants planted in sterilized (8.0 cm) and unsterilized coco-peat (7.5cm) with Th10. Similarly shoot length was more in the plants planted in sterilized (18.8 cm) and unsterilized coco-peat (15.0) with Th10. In control the root length was 5.6 cm and shoot length was 14.4 cm. The vigour index of the plants was <1200 in the pathogen inoculated plants compared to uninoculated control where it was >1500. However in plants that were planted in *Trichoderma* enriched coco-peat, the vigour index was high (1800-2300) even when challenged with pathogen compared to plants raised in coco-peat with *Trichoderma* enrichment (1500-2000).

Bioefficacy tests against Phytophthora capsici *in chillies*

There was a significant reduction in the germination of chilli seeds in the presence of *P. capsici*. There was 50% reduction in germination without *Trichoderma* enrichment while in unsterilized coco-peat with Th10 there was 40% reduction in germination (Fig. 1B). Since the pathogen load was very high and the isolate used was highly virulent, there was high disease incidence in pathogen inoculated treatment. In the absence of pathogen, the germination was 85-90%.

Table 1. Viability of *T. harzianum* propagules in coco-peat in terms of CFUs g^{-1}

Days	Log of CFUs g^{-1} of substrate		
	SCP +Th–10	USCP +Th–10	Mean
7 days	7.50 d	7.08 a	7.29 c
14 days	8.11 a	7.15 a	7.63 a
21 days	7.80 b,c	7.09 a	7.45 b
28 days	7.77 c	6.73 b	7.23 c
35 days	7.84 b,c	6.74 b	7.29 c
42 days	7.83 a,b	6.62 b	7.30 c
Mean	7.83 x	6.90 y	7.36

SCP – Sterilized coco-peat, USCP – unsterilized coco-peat. Values followed by the same alphabets in a column or a row do not differ significantly by LSD. CD @ P=0.05, for sterilization of coco-peat: 0.072, for time of viability test: 0.125, interaction between these two factors: 0.177. At any time of observation the viability in SCP+Th–10 was significantly higher than that of USCP+Th–10. Values followed by letters a, b, c or d in a column do not differ significantly.

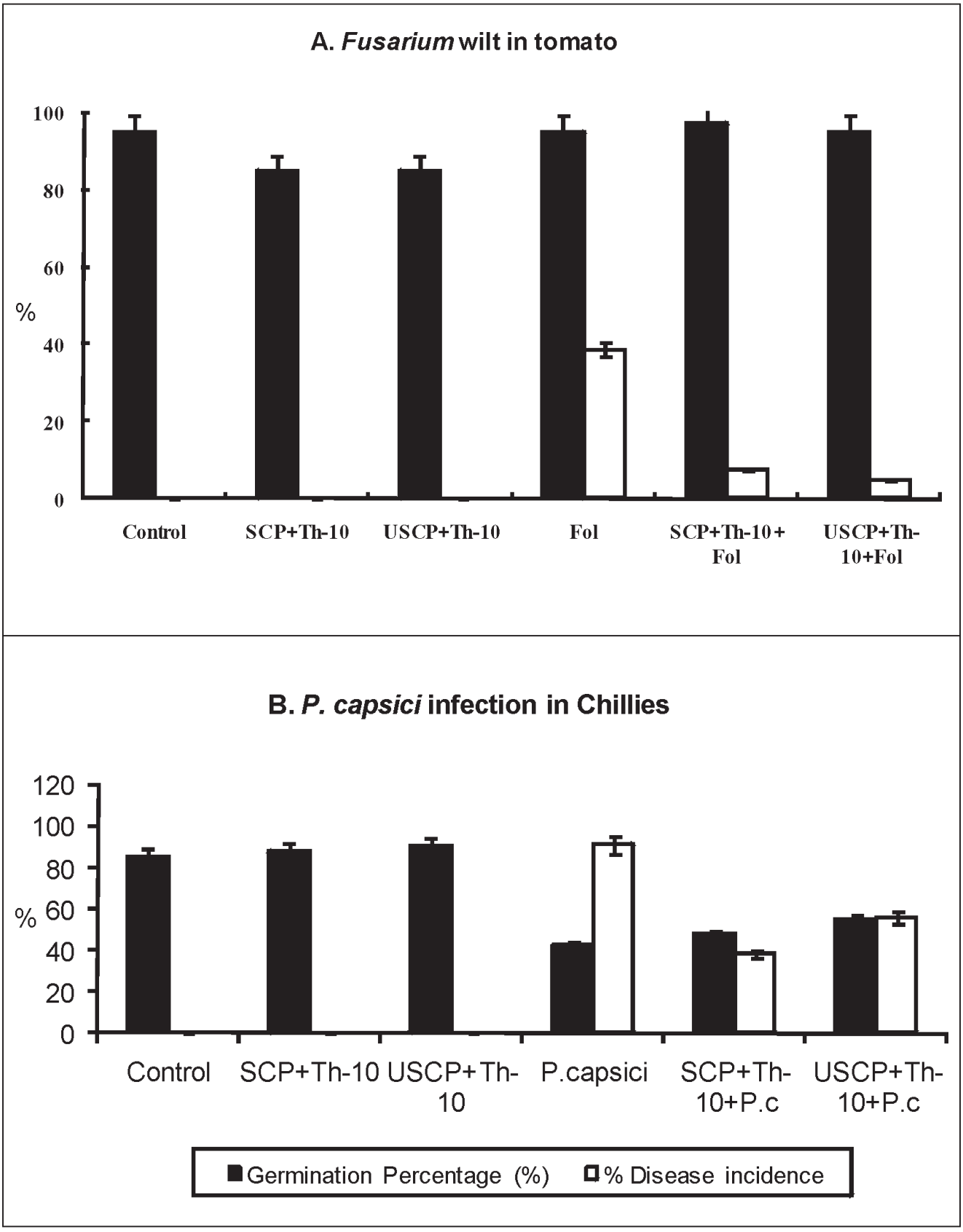

Fig. 1. Effect of sterilizing and enriching coco-peat with *T. harzianum* on seedling germination and incidence of (A) *Fusarium* wilt in tomato and (B) *P. capsici* in chillies. SCP – Sterilized coco-peat, USCP – unsterilized coco-peat, Fol – *Fusarium oxysporum* f. sp. *lycopersici*, P. c. – *Phytophthora capsici*

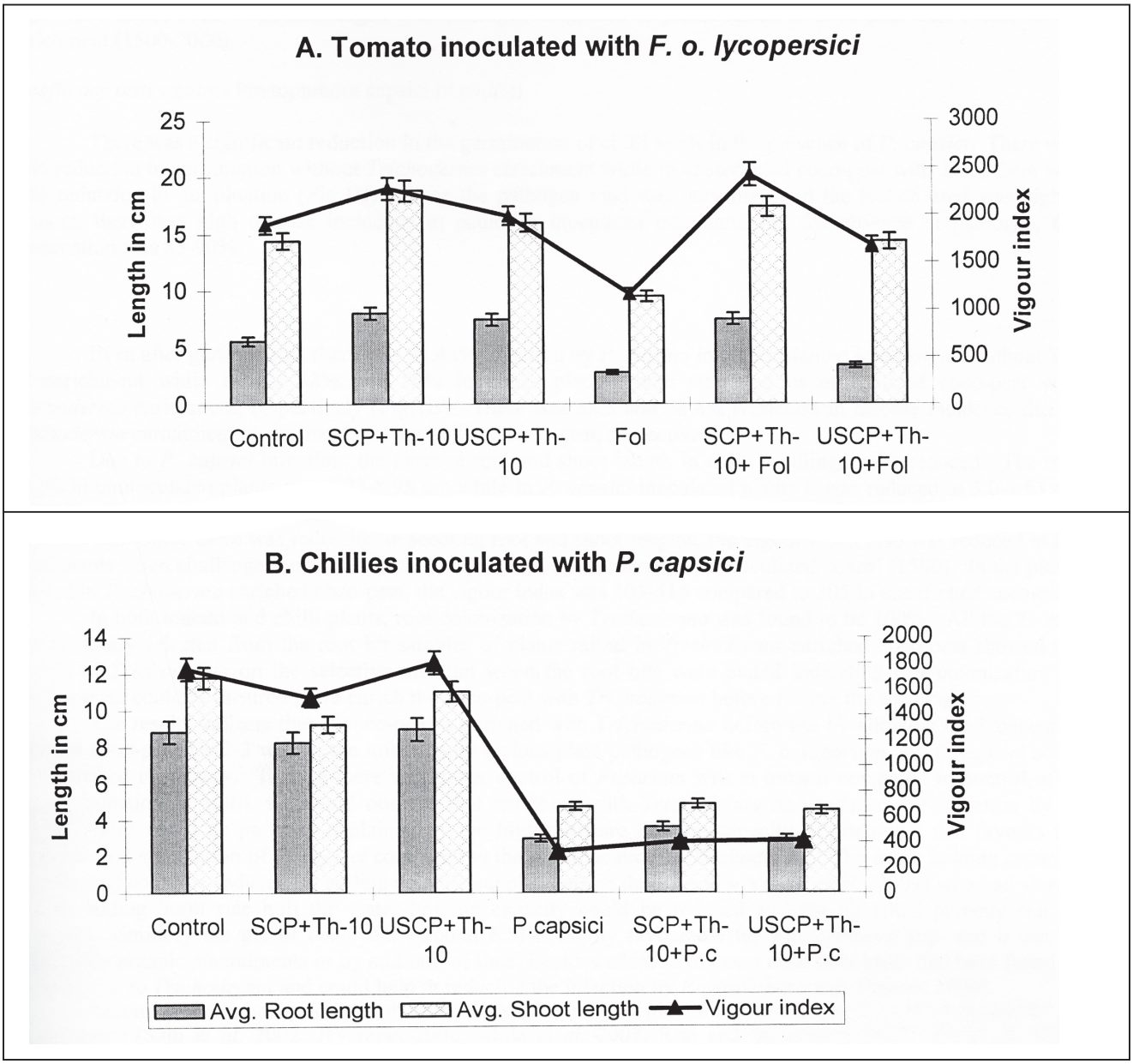

Fig. 2. Effect of sterilizing and enriching coco-peat with *T. harzianum* on growth parameters (root and shoot length, vigour index) in tomato and capsicum plants inoculated with *Fusarium* wilt pathogen and *P. capsici*, respectively. SCP – Sterilized coco-peat, USCP – unsterilized coco-peat, Fol – *Fusarium oxysporum* f. sp. *lycopersici*, P. c. – *Phytophthora capsici*

Even after germination, there was 91.43% infection by *P. capsici* in plants planted in coco-peat without Th-10 enrichment while it was 38% and 56% in plants planted with sterilized or unsterilized coco-peat with *Trichoderma* enrichment, respectively (Fig. 1B). There was 58.3 and 38.8% reduction in disease incidence due to *Trichoderma* enrichment in sterilized and unsterilized coco-peat, respectively.

Due to *P. capsici* infection, the average root and shoot length in chilli seedlings were reduced. The root length in uninoculated plants was 8.23-8.98 cm while in *P. capsici* inoculated plants it was reduced to 3.0-3.63 cm (Fig. 2B). Shoot length in the absence of pathogen was 9.23–11.78 cm while in *P. capsici* inoculated plants it was 4.55–4.9 cm. Since there was reduction in seedling root and shoot lengths, the vigour index also was reduced in the chilli plants when challenge inoculated with *P. capsici* (305) compared to uninoculated conrol (1500). In the plants planted in *Trichoderma* enriched coco-peat, the vigour index was 405-415 compared to 305 in unenriched coco-peat.

In both tomato and chilli plants, root colonization by *Trichoderma* was found to be 100%. All the 25 root bits randomly selected from the root bit samples of plants raised in *Trichoderma* enriched coco-peat showed the growth of *Trichoderma* on the selective medium when the root bits were plated indicating that colonization by

Trichoderma could be ensured if we enrich the coco-peat with *Trichoderma* before raising the seedlings.

The results indicate that if coco-peat is enriched with *Trichoderma* before use by allowing the bioagent to grow on coco-peat for 2-3 weeks, the infection by serious plant pathogens like *F. oxysporium* and *P. capsici* could be controlled in nursery. Though there was better control of *Fusarium* wilt in tomato compared to control of *P. capsici* infection in chilli, we could observe that enriching with *Trichoderma* had reduced the infection by *P. capsici*. This could be partially explained by the high moisture holding capacity of coco-peat that favours the virulence and sproulation of *P. capsici* compared to the multiplication of *Trichoderma*. The water holding capacity of coco-peat could be reduced by adding suitable supplements as described by Yahya *et al.* (2009) who had shown that by adding burnt rice hull the water holding capacity could be reduced and the air filled porosity can be increased. Similarly the pH of coco-peat (around 6.5) is highly favourable for *Phytophthora* spp. and it can be adjusted by organic amendments or by addition of lime. Besides addition of neem cake in coirpith had been found to be beneficial to *Trichoderma* and could help in reducing the infection by *Phytophthora* spp. (Sarma, 2006).

Several workers have recorded the feasibility of using coir-pith as a substrate for farm level production of *Trichoderma* (Saju *et al*., 2002; Jeyarajan 2006; Akila *et al*., 2007; Rini and Sulochana, 2007; Sibi *et al*., 2008; Usharani *et al*., 2008). The results of the present study indicate that colonization of unsterilized coco-peat by *T. harzianum* was not stopped though it was less compared to that of sterilized coco-peat. The coco-peat enriched with *Trichoderma* reduced the *Fusarium* wilt in tomato and *P. capsici* infection in chillies. If commercial nurseries use *Trichoderma* enriched coco-peat, use of chemical fungicides can be minimized and *Trichoderma* that colonizes the root system of seedlings will help in preventing the infection and colonization by pathogens in the main field. Since there is no high additional cost involved if the mother cultures are maintained with low initial input, the use of *Trichoderma* enriched coco-peat can be adopted by commercial nurseries.

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