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Collar rot disease caused by *Rhizoctonia solani* Kuhn in kagzi lime (*Citrus aurantifolia,* Swingle) a new record from the nurseries of Uttar Pradesh and its management.

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Abstract

Collar rot of kagzi lime (*Citrus aurantifolia,* Swing.) is caused by *Rhizoctonia solani* Kuhn. In nursery stage, about 40-55% seedlings of Kagzi lime *(Citrus aurantifolia* Swing*)* were died due to collar rot. This is the first report of collar rot diseases in Kagzi lime caused by *R. solani* from the nursery of Uttar Pradesh. The efficacy of three *Trichoderma* species *viz T. harzianum, T. virens* and *T. viride* against *R. solani* were tested by dual culture technique. *T. virens* and *T. viride* grew very fast before touching the colony of the *R. solani,* thereafter; their growth was significantly slowed down. But *T. harzianum* grew very fast over the colony of *R. solani*, and completely covering it within 8 days. The population of *R.solani* in infested soil could be minimized by treating the soil with *T. harzianum, T. virens* and *T. viride. T. harzianum* and *T. virens* grew very fast and occupied the space and consumed nutrients in the root rhizosphere, thus, outwitted the pathogen.

Key words: *Citrus aurantifolia,* collar rot, *Rhizoctonia solani & Trichoderma* spp.

Introduction

Kagzi lime (*Citrus aurantifolia* Swingle) is an important fruit crop, originated from tropical and subtropical region of South East Asia, particularly India and China. In India citrus crop occupies a prominent place covering an area of about 8.5 L ha with an annual production of 74.64 L tonnes with a productivity of 8.8 t/ha (Anonymous, 2011). Citrus plants and fruits are very susceptible to infestation by fungi, bacteria and viruses (Agrios, 2005 and Singh 2005). From the past two-three years continuously, heavy (42-55%) mortality of seedlings of kagzi lime in the nursery of College of Horticulture, Narendra Deva University of Agriculture and Technology Kumarganj, Faizabad (Uttar Pradesh) has been noticed. In a preliminary survey in many private nurseries in the villages surrounding the university also revealed the occurrence of the similar disease of kagzi lime in nursery beds. The occurrence of such type of disease symptoms on kagzi lime has not been so far reported. Besides, as the disease has reported by Jaganathan (1992) in Tamilnadu and Singh and Andotra (1997) in Jammu and Kashmir. In the field, the disease usually appears in patches. The nursery men attempt to control the disease by treating the seeds usually with PCNB but without success. The maximum percentage germination and survival of kagzi lime seedlings were recorded with benomyl and carbendazim treatment Jaganathan (1992). Singh and Andotra (1997) also reported that soil amendments followed by soil drenching with thiram significantly controlled seedling mortality in kagzi lime. To control soil borne diseases, the use of fungal bio-agents like species of *Trichoderma* has been recommended by many scientists (Papavizas, 1985; Chet, 1987; Harman and Bjorkman, 1998; Harman, 2006). The management of soil borne diseases with *Trichoderma* formulation is eco-friendly, sustainable and cost effective. The present investigation has been carried out with the etiology of the disease and find out the appropriate *Trichoderma* species against the pathogen.

Materials and Methods

The experiment was conducted at Horticulture Research Centre, Narendra Deva University of Agriculture and Technology Kumarganj, Faizabad (Uttar Pradesh), India during 2010-2011.

Isolation of pathogen

The infected damping off or collar rot region of the seedlings were cut into small pieces and surface sterilized by immersing in 0.1 % (w/v) mercuric chloride ($HgCl_2$) aqueous solution for one minute followed by rinsing three times with sterilized distilled water. The surface sterilized pieces were placed in petri plates. The excess water over the seedling pieces was wiped out with three folds of sterilized blotting paper and placed on PDA plates. Finally, these were incubated in BOD incubator at 26 ± 2^{0}C.

Identification of the pathogen

The pathogen was identified by the colony characters and morphological features. For confirmation of the identity, the pathogen was sent to the Indian Type Culture Collection, Division of Plant Pathology, I.A.R.I., New Delhi.

Pathogenicity test of *R. solani*

For pathogenecity test of *R.solani*, five earthen pots (6×7 cm.) were filled with a mixture of garden soil and farm yard manure (FYM) at 1:1 ratio. Finally, chopped tender citrus shoots (50 g/kg soil) were then added to the sub-surface soil of the pots and lightly irrigated to maintain moisture necessary for seed germination. Pot surface was covered with brown paper and sterilized in autoclave at 121.6 °C for 20 minutes. Next day after cooling of the soil, the fungal mat grown in Richard's broth media was mixed in aseptic conditions with the sterilized soil (@10g fungal mat for 500 g sterilized soil). Surface of the pots were again covered with brown paper and incubated in BOD incubator (26 ± 2 °C) for three days. Thereafter, surface sterilized mature seeds of citrus variety Kagzi lime were sown in the fungus inoculated pot soil (@ 10 seeds/pot) and incubated in a growth chamber at 26 ± 2°C. High RH (97%) was maintained inside growth chamber. The pots were lightened for 12 h per day. When the typical symptoms appeared, the pathogen was re-isolated from the infected portions and thus pathogenicity of the fungus was confirmed.

Symptomatology

The symptoms were recorded in details both in naturally and artificially infected seedlings with suitable photographs at different growth stages of the seedlings.

Interaction between the pathogen and the antagonists

The **efficacy** of *T. harzianum*, *T. viride* and *T. virens* against the pathogen *Rhizoctonia solani* (Kuhn) was assessed by using dual culture technique (Morton and Strouble 1955). Five mm disc of each of the antagonists and *R. solani* were cut with the help of sterilized cork borer from the edge of the three days old cultures and were placed over solidified PDA in Petri dishes at 60 mm apart from each other. In control set, only a disc of *R. solani* was placed over the PDA plate. The Petri dishes were incubated in BOD incubator at 26 ± 1 °C. The radial growth of the fungal colonies was recorded every day at 24 h interval till the colonies of *Trichoderma species mowed the colonies of R. solani*. Five replications for each treatment were maintained.

Efficacy of the antagonists against the pathogen *Soil inoculation with R. solani*

To a mixture of garden soil and FYM in equal proportion, finely chopped tender citrus shoots (50 gm/kg.) was added lightly irrigated; pot surface was covered with brown paper and sterilized in autoclave keeping for 20 minutes at 121.6 °C. After cooling of the soil, the inoculums (*R. solani*) grown in solid sorghum media was mixed in aseptic condition with the sterilized soil (@ 50 gm. inoculums for 1.5 kg sterilized soil). Surface of the pots were again covered with the brown paper and incubated BOD incubator (26 ± 2 °C) for five days.

Inoculation of the *R. solani*-infested soil with *Trichoderma* species

When the inoculated pot soil was found to be totally infested with the mycelia growth of the fungus, *R.solani*, as it was evident by the growth of the mycelia over soil surface of the pots, *T. harzianum*, *T. viride* and *T. virens* grown over solid (sorghum) medium for 7 days at 26 ± 2 °C were mixed separately (@ 50 g/pot) with the *R. solani*-infested soil thoroughly.

Population count of *R. solani* and *Trichoderma* species

Population of *R. solani* and *Trichoderma* spp. counted by Soil Plate Dilution technique (Johnson and Curl 1972). After 7 days of inoculation, 200 mg sample from each experimental pot was taken from 10cm depth with sterilized disk cutter. It was put in to 200 ml sterilized distilled water and shaken well, 0.5ml of the suspension was put into sterilized Petri plate into which 30 ml molten and warm PDA and a pinch of Streptomycin were added. The whole mixture was thoroughly mixed and incubated 26 ± 2°C in BOD incubator. To investigate the increase or decrease of population of the *Trichoderma* spp. and *R. solani* with passing time, the experiment was repeated three times at 7 days interval. The colonies of *R. solani and Trichoderma species* in each plate were counted by colony counter. For each treatment five replications were maintained.

Results and Discussion

Identification of the pathogen

Colonies grew fast, usually white to brown grown after 5 days of incubation. The colors of the mycelium was white at the beginning, later turn tan brown with ageing and branched near the distal septum of mother hyphal cell at right angles. Sclerotia were dark brown, round to irregular and consisted of brown and barrel shaped (Alexopoulos *et al.* 2002). On the basis of the above mentioned characters the pathogen was identified as *Rhizoctonia solani* (Kuhn). The identification was further confirmed by I.T.C.C. Centre, IARI (New Delhi) and accession number 6528.

Symptomatology

When the seeds were sown in the heavily *R. solani*-infested soil, the seedling suffered both from pre-emergence rotting and post-emergence collar rot diseases. About 30% seedlings died to pre-emergence rotting. Some of the affected seedlings may come out of soil surface but failed to grow further. The collar rot affected seedlings remained alive for some times. But under highly disease conducive condition none of the seedling survived beyond one month. They either toppled over the necrotic zone at the collar region or became necrotic, black and finally died. The similar symptoms

reported on collar rot disease of sunflower caused by *Rhizoctonia solani* by Lakshmidevi *et al.*(2010).

Pre-emergence rotting

All the seeds in the heavily infected soil were germinated. Usually the seeds germinated by tap roots but sometimes instead of one tap root, 2-3 roots developed from the germinating seeds (Fig. 1. ii, iii, iv and Fig. 2). Tips of the radical underwent rotting (Fig.1. iii, v) resulting into pre-emergence death of the seedlings. In some seedlings at the junction of the rotten and healthy portion of the radical a knot was developed (Fig.1. iv) from where new radical developed and the seedlings managed to emerge out from the soil and remained alive at least for few weeks. In some seedlings tip of the radical underwent necrosis and finally got detached. The upper edge of the cotyledonary leaves turned brown and its dorsal side became black and necrotic (Fig. 1. i). Thus, the partially emerged seedlings finally died due to pre-emergence rooting. The similar symptoms and 74% pre-emergence mortality from infected seedlings of *Pinus caribaea* caused by *Rhizoctonia solani* reported by Narayanappa (1992).

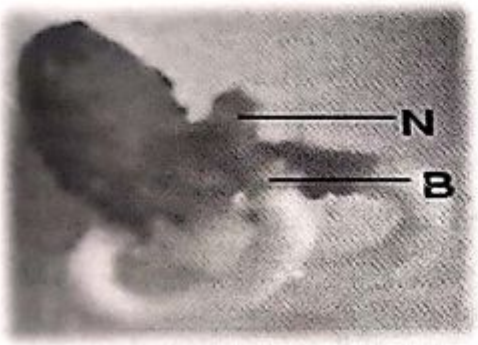

Fig. 1. Pre-emergence infection of citrus seedling by *R. Solani*

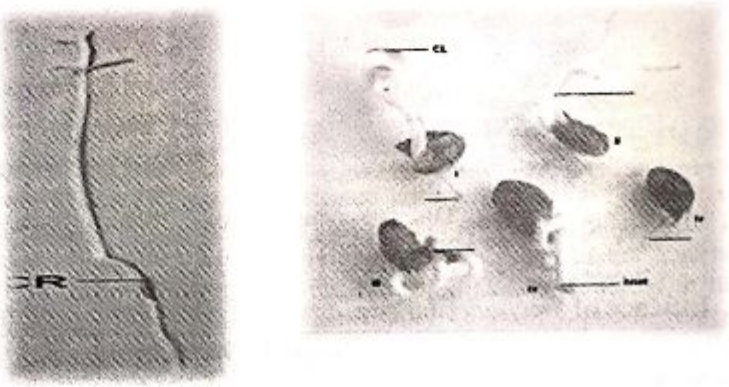

Fig. 2. A citrus seedling infected by *R. solani* at pre-emergence stage (N-necrotic part of the root, B- browing of cells alone the upper part of the necrotic zone).

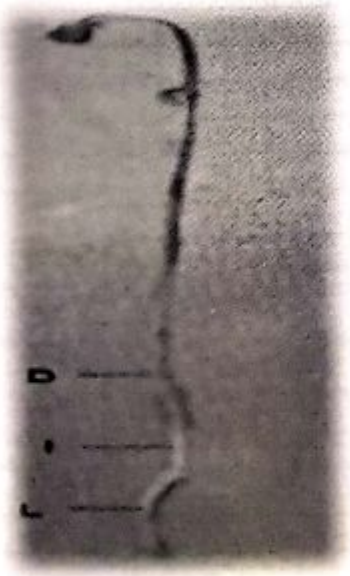

Fig 3. Citrus seedling showing typical symptom

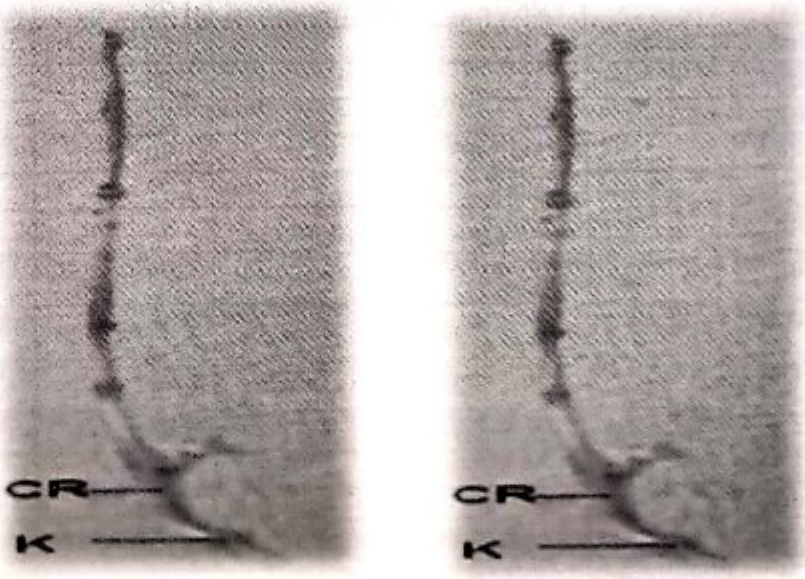

Fig.4. Advance stage of collar rot disease of citrus seedlings. of collar rot disease infected by *R. solani* (CR- collar rot). (L- Living part, D- Dead portion, I- Inner hard tissue of stem, after upper soft tissues underwent necrosis)

Fig.5. Citrus seedling killed by collar rot disease (CR- collar rot, K- knot)

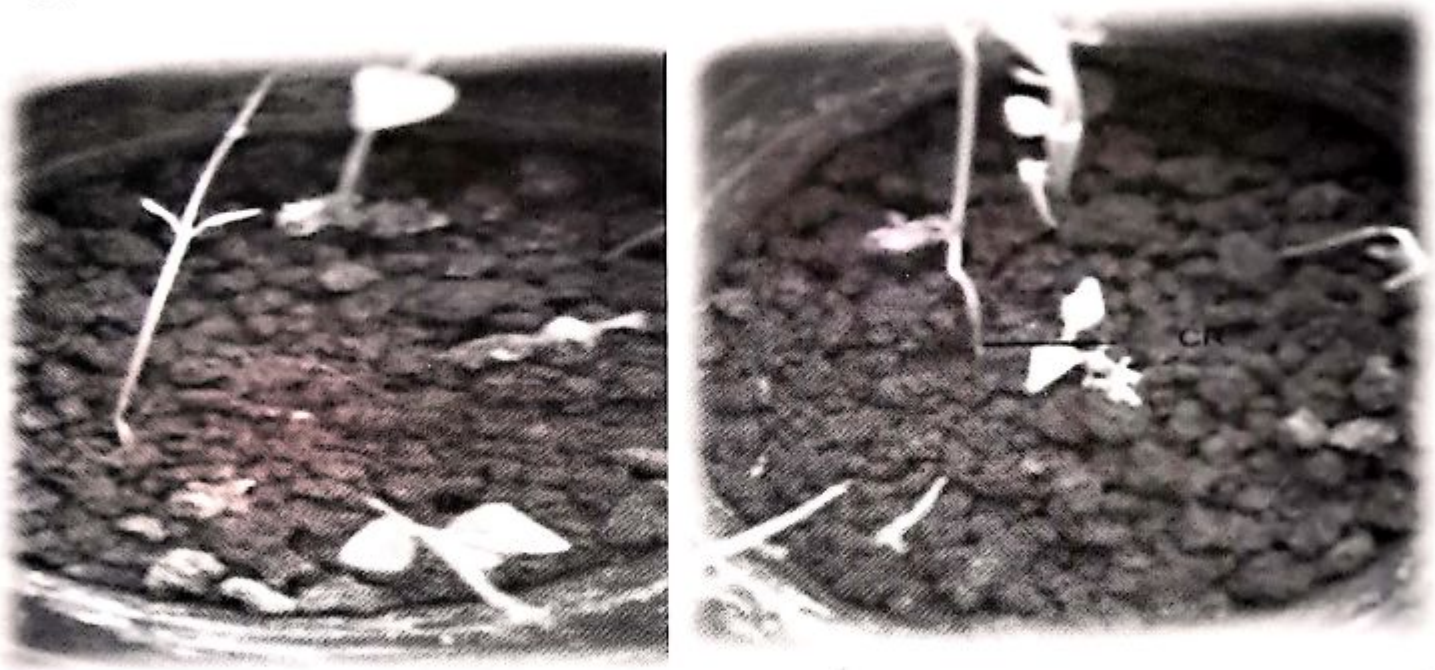

Fig. 6. Diseases symptoms of *R. solani*-infested citrus seedlings

Table 1. Efficacy of *Trichoderma* spp. against *R. solani* (dual culture)

Day of observation (after 24 hour of incubation)	Size of colony (cm) of *Trichoderma* spp.			% decrease of *R. solani* colony/day		
	T. viride	*T. harzianum*	*T. virens*	In *T. viride*	In *T. harzianum*	In *T. virens*
1st.	1.06	0.96	1.44	-	-	-
2nd.	1.28	1.36	1.78	9.01	15.74	16.50
3rd.	1.38	1.44	1.80	4.50	3.73	1.16
4th.	1.46	1.84	1.86	3.77	19.41	3.52
5th.	1.52	2.56	1.88	2.94	43.37	1.21
6th.	1.54	3.14	1.90	1.01	61.70	1.23
7th.	1.62	3.36	1.92	4.08	61.11	1.25
8th.	1.64	3.5	1.92	1.06	100	00
9th.	1.66	-	1.94	1.07	-	1.26
10th.	2.04	-	2.30	22.82	-	23.07
11th.	2.40	-	2.56	24.65	-	61.66
12th.	2.96	-	3.08	50.90	-	55.31
13th.	3.26	-	3.5	55.55	-	100
14th.	3.5	-	-	100	-	-
CD at 5 %	0.14	0.15	0.18			

Efficacy of *Trichoderma* spp. against *R. solani* (dual culture)

Interaction between *R. solani* and *Trichoderma* spp. *in vitro* in dual culture test, the highest growth after 24 h of incubation was recorded with *T. virens* (1.44 cm) followed by *T. viride* (1.06 cm) and *T. harzianum* (0.96 cm). During this period, the colonies of *T. species* and *R. solani* did not touch with each other and the *T.*species also did not develop any

pigment. Next day the same trend was also maintained but differences in the size of colonies between *T. harzianum* and *T. viride* were reduced to significant level. After 72 h, the colonies of *Trichoderma* species and *R. solani* covered in between gap and touched each other. The centre portion of all the *Trichoderma* colonies developed their characteristics green color with white spreading mycelia around the green center. By this time the size of the colony of *T. virens* became maximum (1.80 cm) (Table 1). The second big colony size was recorded with *T. harzianum* (1.44 cm) while minimum with *T. viride* (1.28 cm). Up to 5th day, the same trend was maintained although the differences between *T. harzianum* and *T. viride* were reduced to the significant level. However, on the 6th day the highest growth was recorded with *T. harzianum* (2.5 cm) and minimum with *T. viride* (1.52 cm). Up to the 8th day, the same trend was maintained. During this period, *T. harzianum* was found to engulf the entire colony of *R. soloni*. Hence, further recording of colony size of *T. harzianum* was stopped. *T. harzianum* controlled the disease caused by *R. solani* due to its fast growth (Sawant *et al.*, 1995). *T. virens* became successful to over grow the colony of *R. solani* on 14th day. *T. viride* took one day more time to completely smoothed the colony of *R. solani*. *This finding supports the results of* biological control agent *Trichoderma viride* MNT-7 reduced the collar rot disease caused by *R.solani* (Ramesh, 2002).

Interaction between *Trichoderma* species and *R. solani* in soil

The number of colonies of *T. harzianum, T. viride, T. virens* and *R. solani* both in the treated and control soil have been presented in the Table 2. The number of colonies of *T. harzianum, T. viride* and *T. virens* in the treated soil seven days after the treatment was recorded as 2.7×10^4, 5.1×10^4 and 5.8×10^4 colonies/g-soil respectively. However in the untreated soil the colonies were counted 1.1×10^6. Number of *T. harzianum* colonies in the same soil was 6.5×10^4 colonies/gm soil. After days of treatment, there was a reduction in number of colonies of *T. harzianum*. But on twenty-one days after the treatment, the population of *T. harzianum* was again found to be increased. On the other hand, the colonies of *R. solani in* the treated soil showed a gradually decline. In artificially infested soil, *T. harzianum* reduced the population of *R. solani* and protected sugarbeet seedlings from damping-off in three weeks after inoculation (Kok *et al.* 1996). In the *T. viride* treated soil, the population reduction was recorded after fourteen days. But within the next seven days, the population was again built-up and it reached the peak. The population of *R. solani* both in treated and untreated soil recorded a gradual reduction from seven days after treatment up to the end of the experiment. However, rate of reduction of population of *R. solani* in treated soil was considerably higher, but population level was just reverse and the population of *T. virens* from seven days to twenty-one days of the treatment showed a marginal decline while that of *R. solani* in the treated soil reduced significantly. Contrarily, population of *R. solani* in untreated soil was considerably higher than the treated soil. The similar result were reported by Kavitha *et al.* (2006) that *Trichoderma viride* on dry root-rot of *Citrus aurantiifolia* caused by *Fusarium solani* was reduced the disease incidence equally and significantly.

Table 2. Population of *R.solani* and *Trichoderma spp.* in *R. solani*-infested soil (colonies/g soil)

No. of days after treatment	The fungal population in *T. harzianum* treated soil		The fungal population in *T. harzianum* treated soil		The fungal population in *T. harzianum* treated soil		Colonies of *R. solani* in untreated (control) soil
	T. harzianum	*R. solani*	*T. viride*	*R. solani*	*T. virens*	*R. solani*	*T. harzianum*
7	6.5×10^4	2.7×10^4	5.1×10^4	3.3×10^4	5.8×10^4	3.0×10^4	1.1×10^6
14	4.6×10^4	2.2×10^3	4.6×10^4	1.1×10^3	5.6×10^4	2.1×10^3	4.6×10^5
21	6.2×10^4	1.8×10^2	5.9×10^4	1.5×10^2	5.5×10^4	1.7×10^2	6.2×10^5

Table 3. Per cent reduction of *R. solani* colony over the control in *Trichoderma* amended soil

No. of days after treatment	Per cent reduction of *R. solani* colony over the control		
	T. harzianum	*T. viride*	*T. virens*
7	75.22	69.72	72.48
14	66.15	83.08	67.69
21	73.13	77.61	74.63

Efficacy of the *Trichoderma* species in reducing the population of *R. solani*

The per cent reductions in *R. solani* colony over the control after the treatment with the three species of *Trichoderma* are presented in the table 3. With *T. harzianum* the initial reduction was 75.22%. But in the next seven days the per cent reduction was reduced to 66.15%. However at the end of experiment the per cent reduction again increased and attended the level of 73.13%. With *T. viride* the reduction after seven days of treatment was the minimum (69.72%) of all the three treatments. But, within the next seven days, *T. viride* increased its reduction percentage to 83.08%. *T.virens* showed the percent reduction of 72.48%, 67.69% and 74.63% at 7,14 & 21days after the treatment respectively. The presence of *T. harzianum* population density of *R. solani* was reduced significantly (Paula and Hau, 2007). Thus, according to the initial efficacy the *Trichoderma species* may be arranged in the following sequence: *T. harzianum* > *T.virens* > *T. viride.* But at the end of the experiment a reverse picture was noticed like this: *T. viride* > *T.virens* > *T. harzianum.*

Conclusion

The infection of *R. solani* in the citrus nursery took heavy loss. The symptoms of *R. solani* were manifested either in the form of pre-emergence rotting or collar rot of young seedlings. The infected seedlings fought back the pathogen by producing more roots or developing mechanical barrier in the form of knot separating the infected zone from the healthy one to stop the progress of the pathogen from infected root tips to the upper part. The population of *R.solani* in infested soil could be minimized by treating the soil with *T. harzianum, T. virens* and *T. viride. T. harzianum* and *T. virens* grew very fast and occupied the space and consumed nutrients in the root rhizosphere, thus, outwitted the pathogen. On the basis of the results observed in the present experiment, application of *T. viride* and *T. virens* as preventive measures and that of *T. harzianum* as curative one may be suggested.

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