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Effect of *Trichoderma* sp. on *Sclerotium rolfsii*, the Causative Agent of Collar Rot on *Zamioculcas zamiifolia* and an on Farm Method to Mass Produce *Trichoderma* species

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Abstract: The antagonistic effect of three local isolates of *Trichoderma viride* and one local isolate of *Trichoderma harzianum* were tested against the pathogenic fungus *Sclerotium rolfsii*. The latter organism is responsible for major loss due to collar rot of the ornamental crop *Zamioculcas zamiifolia* in Sri Lanka. The disease causes massive losses. The antagonistic potential of the local isolates against the phytopathogenic fungi *Sclerotium rolfsii* was investigated in dual culture, poison food technique, pot trials and field trials on *Zamioculcas zamiifolia* plants. All *Trichoderma* isolates tested under *in-vitro* conditions significantly inhibited the growth of *S. rolfsii*. Of these isolates, *Trichoderma viride* isolate Tv1, showed highest percentage inhibition and was thus selected for *in vivo* field trials. Data recorded from bi monthly field application of this organism over the two growing seasons, confirmed the success of the treatment in controlling collar rot disease at the economic threshold level. Field application of testing isolate *T. viride* Tv1 as a conidial suspension (10^{11} cfu mL^{-1}) greatly reduced the disease incidence of *Zamioculcas zamiifolia* plants by a percentage of 75.54%. On farm mass production of this isolate was developed to help facilitate the establishment of an integrated eco-friendly disease management system for growers of *Zamioculcas zamiifolia*. Different media was also evaluated to mass produce the *Trichoderma* isolate. The media evaluated in this study included the solid substrates barley seeds, paddy, cow pea, maize and sorghum and semi solid, liquid substrates such as potato dextrose, rice extract, paddy extracts, respectively. Although mycelial growth was fastest in barley and paddy media. And the highest yield of spores of the *Trichoderma* isolate was observed 7 days after inoculation in Barley and Paddy media.

Key words: *Trichoderma viride*, *Trichoderma harzianum*, *Sclerotium rolfsii*, antagonism

INTRODUCTION

Zamioculcas zamiifolia is an important plant grown in Sri Lanka for export. *Zamioculcas* is the genus of flowering plant in the family Araceae, containing the single species *Z. zamifolia*. It is a tropical perennial, herbaceous plant growing to 45-60 cm tall, from a stout underground, succulent rhizome. It is normally evergreen, but becomes deciduous during drought, surviving drought due to large potato-like rhizome that stores water until rainfall resumes. The leaves are pinnate, 40-60 cm long, with 8-10 pairs of leaflets 6-10 cm long: they are smooth shiny and dark green. The export quality of *Z. zamiifolia* declined due to the repeated occurrence of a collar rot caused by *Sclerotium rolfisii*. The collar rot disease cycle begins with the germination of sclerotia. Mycelium fans out in all directions from the sclerotia, slowly growing across the surface of the soil in warm, wet weather. Symptoms begin to appear on *Z. zamifolia* after period of prolonged hot, humid weather. The lower leaves turn yellow, then brown and finally wilt from the margins back toward the base. The upper leaves soon collapse. Widespread occurrence of the collar rot affected *Z. zamiifolia* production causing a significant setback to the floriculture industry in Sri Lanka. Consumer concern has made it necessary to develop eco-friendly methods to control this disease. Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods and also found that many isolates of *Trichoderma* species produced non-volatile antibiotics, which were active against a range of fungi (Eziashi *et al*., 2007). One such method is the use of biological controlling agents such as *Trichoderma*. The bioagent, *Trichoderma* species are known antagonists of other fungi and have been shown to be very efficient biocontrol agents of several soil borne plant pathogenic fungi (Barakat *et al*., 2006; Karthikeyan *et al*., 2006).

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Trichoderma sp. is known mycoparasites of a number of plant pathogens. *T. harzianum* colonizes *S. rolfsii* hyphae, disrupts mycelial growth and kills the organism. *Trichoderma* has the ability to suppress the growth of many pathogenic fungi (Chet *et al*., 2009; Mukhopadhyay, 2009). It has also been reported that *Trichoderma* species with different mechanism such as lysis of sclerotia, inhibited mycelial growth of *S. rolfsii* with volatile metabolites producing and parasitized the hyphal trends of disease agent (Shaigan *et al*., 2008).

Developing appropriate formulation and delivery systems is the pre-requisite for implementing biological control using microbial antagonists and the formulation of biological control agents depends upon biomass production and maintaining viability at the end of the process (Thangavelu *et al*., 2004). The aim of this study was to evaluate the efficacy of *Trichoderma* species to control collar rot in *Z. zamiifolia* plantation under field conditions. In this investigation antagonistic effect of three isolates of *Trichoderma viride* and one isolate of *Trichoderma harzianum* were tested against *Sclerotium rolfisii*.

MATERIALS AND METHODS

The experiment was carried out under field condition in the growing seasons of 2006, 2007 and 2008 in soil naturally infested with *S. rolfsii* in an Ornamental foliage nursery located in the low country intermediate zone of Sri Lanka.

Isolation of fungi

***Trichoderma* sp.:** The *Trichoderma* species used in this study were isolated from soil samples obtained from Green Farms Ltd, Marawila, Sri Lanka using the soil dilution technique (Subba, 2003). Pure cultures of the *Trichoderma* isolates were maintained on PDA at 25±2°C and the isolates were identified using morphological and reproductive characters (Anonymous, 2006; Bisset, 1991; Lieckfeldt *et al*., 1999; Samuels *et al*., 1998; Watanabe, 2002a).

***Sclerotium rolfsii*:** Infected *Z. zamiifolia* plants were uprooted from the field at Green Farms Ltd and were immediately brought to the laboratory. The plants were kept in moist polythene bags to enhance mycelial growth prior to isolation of pathogen on Potato Dextrose Agar (PDA) medium. The fungus was identified based on morphology and colony characteristics (Sarama *et al*., 2002; Punja and Damini, 1996; Harlton *et al*., 1995; Watanabe, 2002b) The pathogenicity of the isolated *S. rolfsii* was established by following the Koch's postulates (Riley *et al*., 2002) as described below.

Sclerotium rolfsii grown in pure culture was used to inoculate *Z. zamiifolia* plants through topical application to the basal portion of the stem. Six months old *Z. zamiifolia* were transplanted in polythene bags. Spore suspension of the fungus was prepared in distilled water using 7- day- old cultures grown on PDA. The culture was flooded with 10 mL sterilized distilled water and then scraping the culture surface was scraped with a sterile glass rod. (Hong and Hwang, 1998). After filtering the resulting suspension through double layers of cheese cloth, the spore concentration was adjusted using sterile distilled water to 10^4 spores mL^{-1} (Hong and Hwang, 1998). Base of the stem of the plants at soil line was pricked by a blade and the *S. rolfsii* spore suspension was smeared on the injured portion. Occurrence of symptom of collar rot was observed daily.

Antagonistic effects of *Trichoderma* sp.

Dual culture technique: *Sclerotium rolfsii* and the test *Trichoderma* isolate was inoculated at the center of two parallel radial lines on a 9 cm diameter PDA plate. The fungi for inoculation were obtained from the margin of actively growing 7-day old cultures on PDA. The radial mycelia growth of the *Trichoderma* sp. and *S. rolfsii* were measured daily for 7 days. The treatments were replicated five times. Control was with *S. rolfsii* alone (Singh *et al*., 2004). This trial was repeated three times. The percentage growth inhibition (I) was calculated using the formula given below (Datta *et al*., 2004):

$$I\ (\%) = \frac{C\text{-}T}{C} \times 100$$

Where:
I = Percentage inhibition of pathogen by antagonists
C = Radial growth in control
T = Radial growth in the treatment

Poison food technique: Poisoned food technique (Bhanumathi and Ravishanker, 2007) was followed to determine the inhibitory effect of *Trichoderma* isolate Tv1 on *S. rolfsii*. Conidia suspensions (1 mL) of *Trichoderma* isolates prepared as described below was poured into a sterilized Petri dish followed by 15 mL of PDA. One milliliter of distilled water was used instead of the conidia suspension in the control. Four mm diameter discs were obtained from the actively growing region of a 7-day old *S. rolfsii* culture on PDA and were transferred aseptically to the center of each *Trichoderma* isolate amended PDA medium. The treatment were replicated five times in a completely randomized design and repeated three times. The Petri dishes were incubated at 28±2°C and 72±4% RH.

Growth of *S. rolfsii* was determined at 3 and 7 days after inoculation by measuring the mycelial growth diametrically.

Preparation of *Trichoderma* conidia suspensions: Conidia suspension of the test isolates of *Trichoderma* was prepared from seven-day- old cultures on PDA. A 9 cm diameter PDA plate was flooded with 10 mL sterilized distilled water and shaken for a few minutes. The resulting suspension was filtered through muslin cloth (Hong and Hwang, 1998) and the conidia concentration of the filtrate was adjusted to 10^4 spore's mL^{-1} using sterilized distilled water.

Effect of *T. viride* on sclerotia of *S. rolfsii*: Among the *Trichoderma* isolates, *T. viride* Tv1 which showed the highest percentage inhibition (*in-vitro*) was selected for this trial. Mycelia of *S. rolfsii* having sclerotia were collected from naturally infected *Z. zamiifolia* plants and were transferred to the laboratory. The samples were surface sterilized with 1% sodium hypochlorite solution for 2 min. Thereafter, 1 cm diameter discs were taken from the samples and were immersed in a conidia suspension of *T. viride* Tv1 prepared as described above but having 1×10^{11} conidia mL^{-1} for 10 min and placed on a PDA plate (Henis *et al*., 1983). In the control the pathogen samples were immersed in sterilized distilled water for 10 min. The germination of sclerotia was determined. Five replicates were used with three Petri plates per replicate.

Preparation of *Trichoderma* inocula for field tests

Mass production of *Trichoderma*

Solid media: Paddy soaked in water for 6 hours was parboiled in a pressure cooker (1.1 kg cm^{-2} pressure for 45 min) having sufficient amount of water. After parboiling, the closed container was kept in, a cooler room (15±2°C) for 2 h. Five kilogram of the parboiled paddy was equally distributed among 50 polyethylene bags. Mouth of the bag was passed through a polyvinyl pipe of 2 cm diameter and 0.6 cm width and the mouth was thereafter plugged with a piece of sterilized, non absorbent cotton. A piece of paper was wrapped over the cotton plug and the paper was kept intact using a rubber band. The same procedure was followed with other grains - barley, maize, sorghum, white pericarp cowpea and brown pericarp cowpea.

Liquid media

Paddy extract: The extract water resulting from parboiling described above was used as liquid medium to grow *Trichoderma*. This extract was dispensed in flat 250 mL bottles (each bottle having 50 mL of extract) and was sterilized.

Rice extract: Rice (250 g) was cooked by adding 2 L of water. Once the rice was cooked the excess water was drained off and 50 mL of this water was dispensed in flat bottles of 250 mL. The bottles were sterilized thereafter.

Semi-solid media: Potato (200 g) was cut into small pieces, boiled in 1 L of water and filtered through muslin cloth and 20 g of dextrose was added to the filtrate. The suspension was made up to 1 L and 50 mL was poured in to flat bottles of 250 mL and sterilized.

Inoculation of media: Plugs of 4 mm diameter obtained from pure cultures of 7-day old *Trichoderma* isolates on PDA were used to inoculate the above media.

Growth of the fungus: The growth of the fungus was determined by measuring the conidia yield in 1.0 mL or 1.0 g of the culture after appropriate serial dilutions at 7 and 14 days after inoculation. A double ruled Neubauer's haemocytometer was used to count the conidia. Initiation and cover of medium with mycelium was also observed daily. Four replicates were used for each treatment.

Pot trials: Stem cuttings (30 cm) of *Z. zamiifolia* were surface sterilized by immersion in 0.1% aqueous sodium hypochlorite for 2 min and thoroughly rinsed in sterile distilled water prior to being rooted in 12 cm pots containing sterile, moist coir dust. Pots were kept in propagation beds for 3 weeks. After the cuttings rooted they were carefully taken from the pots and repotted in a mixture of compost + coir dust (1:1) at a density of two rooted cuttings per 19 cm pot. Plants were fertilized with the fertilizer mixture (N-P-K, 12-11-18). With the application of the fertilizer, the pH of the media was adjusted to 4.8-5.3 and the electrical conductivity was adjusted to 1.8 mS.

The resulting six -weeks old *Z. zamiifolia* seedlings were inoculated with a conidial suspension of Tv1 (10^{11} conidia mL^{-}), as close as possible to the root system (2-3 cm) with a sterile syringe. Control plants were treated similarly but with sterile water only. Four days later, plants were inoculated by introducing two plugs (5 mm diameter) of actively growing mycelium of S. rolfsii obtained from a 7-day old on PDA, as close as possible to the root system (3-5 cm). Controls were treated with fungus-free PDA disks. The experimental design included the following treatments: (1) Controls (2) Tv1 only, without S.rolfsii, (3) *S. rolfsii* only, without Tv1 and (4)

Tv1 and *S. rolfsii* both. Twenty-five plants were used for each treatment and the experiment was repeated twice. Five replicates and five plants in each replicate. After 5 days the stem were pulled out of the substrate and examined for fungal infection (visible necrotic lesions). Samples from the stem (color) were collected 5 to 7 days after pathogen inoculation and either inoculated on PDA or processed for microscopy.

Field trials: The most promising antagonistic *T. viride*, Tv1 was also used for field trails. A six month old *Z. zamiifolia* plantation naturally infected with *S rolfisii* was selected as the experimental area. *Z. zamifolia* were in field plots having 12 bushes m^{-2}. The size of a plot was 1×30 m^2. Treatments involving soil and foliar applications of *T. viride* Tv1 isolates and the untreated control. Treatments were replicated five times. They were in randomized completely block designs. *T. viride* Tv1 was mass cultured on sterilized parboiled paddy as described above (section 2.3.1.1 and 2.3.2). *T. viride* Tv1 formulations were prepared as follows.

Liquid formulation: One kilogram of 7-day-old *Trichoderma viride* Tv1 cultures on parboiled paddy was flooded with 2 L of tap water and shaken well in a closed container. The resulting suspension was filtered through muslin cloth. The conidia concentration of the filtrate was adjusted to 10^{11} cfu mL^{-1} using tap water. The number of conidia was determined by counting in a double ruled Haemocytometer. One liter of this *T. viride* Tv1 conidia suspension was mixed with 1 mL surfactant (Lankem Ltd, Colombo, Sri Lanka) before being applied as a foliar spray.

Powder formulation: One kilogram of 7-day-old mass cultures was mixed with 500 g of Talc powder (W.H. HENDRICK and SONS LTD. Colombo 11, Sri Lanka) by using a mixing machine (Green Farms Ltd) for 1 h (22 rpm). Talc mixed paddy was sieved through 2×2 mm GI wire mesh. The conidia concentration of the extract was adjusted to 10^{11} CFU g^{-1} with Talc powder. This powder formulation was mixed with cow dung (1:5) (Sangle and Bambawala, 2004) before application.

Method of applications: In foliar applications, each plant (i.e., plant with 2-3 suckers) was sprayed with 200 mL of *T. viride* Tv1 conidia suspension prepared as described above. The plants were sprayed with the above Tv1 conidial suspension until runoff at 2 to 4 weeks intervals. The plants were sprayed with irrigation water until runoff in the control plots. The cow dung mixed powder formulation was also applied as a top dressing at 2 to 4 weeks intervals together with foliar applications at the rate of 100 g m^{-2}. This mixture was distributed by hand as uniformly as possible over the bed area. Time of the application of *T. viride* Tv1 was done based on the collar rot disease development rate records. However, some of the applications were applied at weekly intervals when disease severity became very high (Lo *et al*., 1997). In both methods of applications cultural practices such as field sanitation and manual removal of infected plants were strictly followed throughout the experimental period. Further 40 g of fertilizer mixture (N%- P_2O5%- K_2O% 11 -12-14) per one square meter was applied at 2 weeks intervals.

Measurement of disease: Disease assessments were recorded at two weeks intervals in both this treated plants and the untreated controls up to the end of the trials. The assessments were carried out according to the Horsfall-Barratt rating scale given below (Egel and Harmon, 2001; Horsfall and Barratt, 1945).

Rating scale 1-5:

- 0% disease = 1
- 1-10% disease = 2
- 11-25% disease = 3
- 26-50%disease = 4
- >50% disease = 5

Rainfall, relative humidity and soil PH/EC were also measured through out the study period. Percentage Disease Control (PDC) was calculated by using the following equation described by Engelhard (1997)

$$PDC = \frac{DI_{ck}-DI_{tr}}{DI_{ck}}\times100$$

DI_{ck} = Mean disease incidence in check plot
DI_{tr} = Mean disease incidence in treated plot

The effect of the transformation is to relate the efficacy of candidate material to that of control. When PDC is 100, infection is not present in treated plot. When PDC is zero treated plot had the same level of infection as the check plot.

Study of growth parameters with *T. viride* Tv1 and Un-treated control.

At the end of the experiment 50 samples were randomly taken in different treatments of each replicates separately. Then growth parameters (height of plant in cm and weight of roots/shoots in g and number of suckers/bush) were measured.

Experimental design and data analysis: *In-vitro* experiments were arranged as a complete randomized design with five replicates. All pot experiments and field experiments were established as a randomized complete block design with five replicates. All data were analyzed by one-way ANOVA, Differences among the means were evaluated for significant according to Turkey's pair wise comparisons test ($p<0.05$) (SPSS scientific software and mini-tab software were used for processing the data).

RESULTS AND DISCUSSION

Isolation of *Trichoderma*: Four different forms of *Trichoderma* were isolated from the soil samples obtained from Green Farms Ltd Marawila, Sri Lanka. They were *T. viride* Tv1, *T. viride* Tv2, *T. viride* Tv3 and *T. harzianum* Th1.

Effect of *Trichoderma* isolates on *S. rolfsii*

***In-vitro* tests:** In Dual culture technique and poisoned food technique all isolates of *Trichoderma* suppressed the growth of *S. rolfsii*. The isolates *T. viride* Tv1 showed the highest suppression (Fig. 1) (Johnson *et al*., 2008).

Mass production of *Trichoderma* sp.: The highest mycelia growth was observed in the Barley and Paddy media (Table 1). The highest number of conidia was also produced in these two media. In the semi-solid potato dextrose medium though the growth was high sporulation was low. Hence Paddy medium was used to mass produce *Trichoderma*.

Pot trials: Treatment with *T. viride* Tv1 prior to *S. rolfsii* inoculation resulted in a significant decrease in the occurrence of the disease. In the absence of Tv1 treatment the seedlings exhibited typical symptoms as early as 5-days after inoculation (Table 2). Further in all such infected plants stem damage was always associated with the presence of *S. rolfsii*.

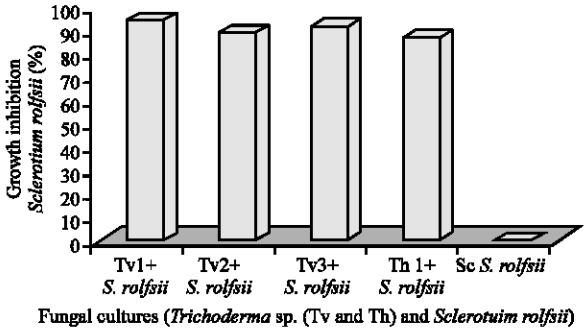

Fig. 1: Growth inhibition (%) of *S. rolfsii* by *Trichoderma* isolates

Field trials: The combined application of *T. viride* Tv1 conidia suspension having 0.1% surfactant and the powder formulation having cattle manure reduced the severity of the collar rot disease (Fig. 2a, b, Table 3).

***In-vitro*:** The results revealed that the variation of antagonistic potential between isolates was due to the variation in mycelium-coiling rate, sporulation, fungitoxic metabolites, induced growth response and temperature effect (Barkat *et al*., 2006). The results showed that Tv1 was the most effective isolate inhibited *S. rolfsii* mycelial growth.

In dual culture technique all the isolates had the ability to parasitize the mycelium of *S. rolfsii*. Microscopic examination revealed the formation of coils around the hyphae of *S. rolfsii* by *Trichoderma* isolates, causing lysis of the hyphal walls. Such observation has been reported by many workers (Mukherjee and Raghu, 1997).

Table 1: Growth and spore yield of *T. viride* Tv1 in different substrates

Substrate	Mean days for the complete coverage of the medium	Spore yield 7 days after inoculation (in 1 g or 1 mL)
Barley	3.00±0.25	4×10^{18}
Paddy	3.75±0.5	3×10^{18}
White pericarp cowpea	4.00±0.25	3×10^{14}
Red pericarp cowpea	4.00±0.25	6×10^{15}
Maize seeds	7.50±0.5	2×10^{10}
Sorghum seeds	9.50±0.5	4×10^{9}
Potato dextrose	3.00±0.25	5×10^{7}
Rice extract	5.33±0.2	2×10^{6}
Paddy extract	5.00±0.5	3×10^{7}

Table 2: Effect of *T. viride* Tv1 on the growth of *S. rolfsii* in the pot trials under field conditions

	Average disease occurrence (10 days after inoculation)				
Treatments	R1	R2	R3	R4	R5
Control	0	0	0	0	0
Tv1 without *S. rolfsii*	0	0	0	0	0
S. rolfsii without Tv1	3	5	4	5	4
Tv1 and *S. rolfsii*	1	0.5	1	1	1.5

Table 3: *S. rolfsii* collar rot disease severity with *T. viride* Tv1 treatment and un-treated control

	Mean collar rot disease severity±SD			
Year	*T. viride*	Control	F-value	p-value
Year 2006/2007				
Season 1	1.51±0.27	5.08±0.44	296.16	0.01
Season 2	0.62±0.18	4.49±0.60	252. 27	00.0
Year 2007/2008				
Season 1	1.35±0.18	4.58±0.65	174.28	0.02
Season 2	0.64±0.19	5.1±0.89	174.34	0.00

Data are means of five replicates at two weeks intervals. Disease severity represents the percentage of the total number of plants that contained diseased plants per replicates at two weeks interval

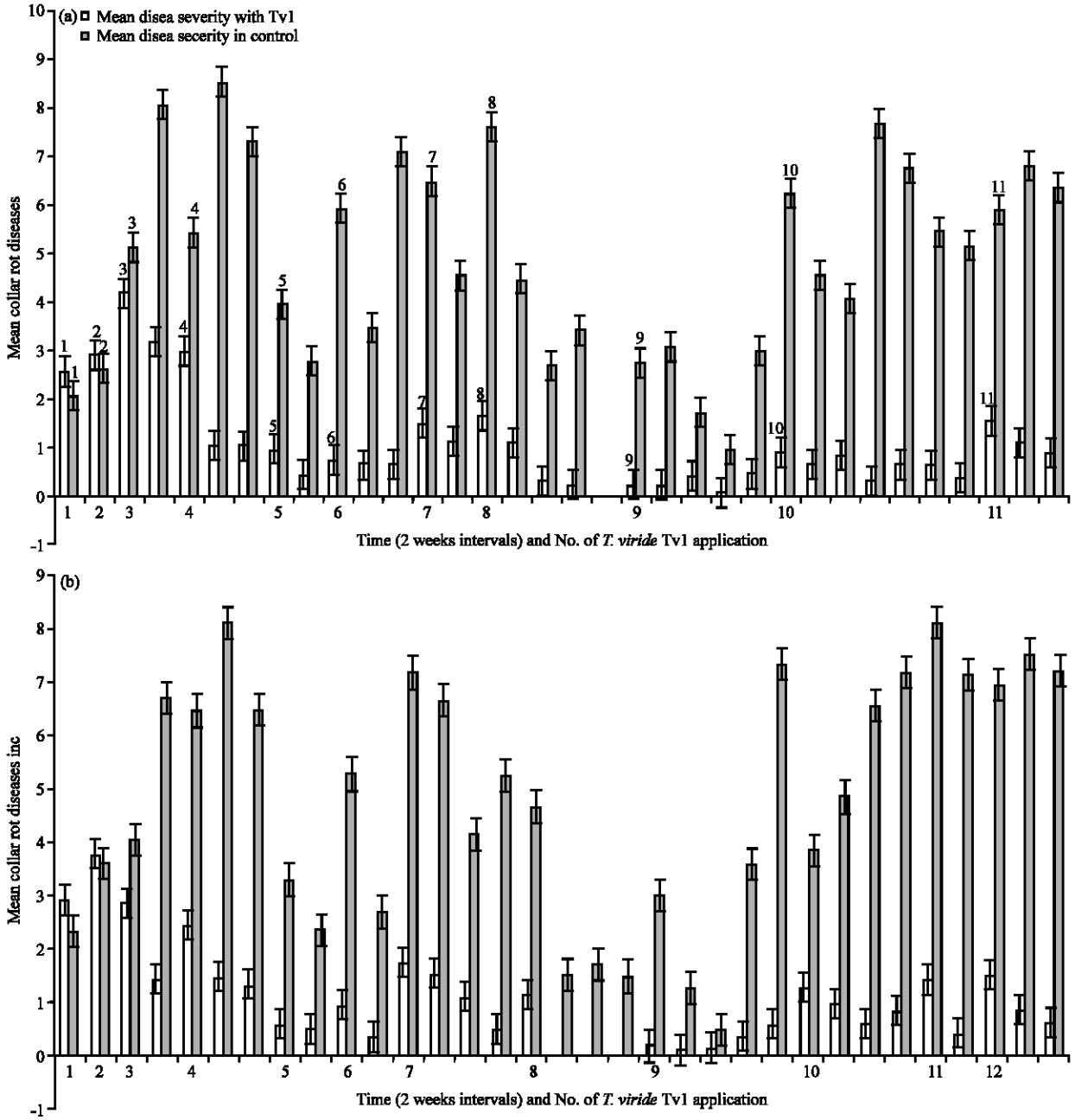

Fig. 2: (a) Mean collar rot Disease severity with *T. viride* Tv1 treatments (both soil and foliar application together). Year 2006/2007. Disease severity represents the percentage of the total number of plants that contained diseased plants per replicates at two weeks interval. Data are means of five replicates at two weeks intervals and (b) Mean Disease severity of Collar rot disease with *T. viride* Tv1 treatment (both soil and foliar application together) Year 2007/2008. Disease severity represents the percentage of the total number of plants that contained diseased plants per replicates at two weeks interval. Data are means of five replicates at two weeks intervals

Since the *T. viride* Tv1 appears to be the most efficient isolate, *T. viride* Tv1 was used for further trials in this investigation.

In the poisoned food technique the conidial suspension of Tv1 completely suppressed the growth of *S. rolfsii*. This was also due mainly to formation of coils around the hyphae of *S. rolfsii* by Tv1. The sclerotia of *S. rolfsii* failed to germinate when inoculated with Tv1. After 14 days, sporulation of Tv1 on the sclerotia was observed. The sclerotia lost their rigidity, darkened and

finally degraded after about 4 weeks. In the control the sclerotia germinated profusely. Some researchers have expressed doubts about the ability of *Trichoderma* to parasitize healthy sclrotia (Henis *et al*., 1983). The results of this investigation clearly indicate that *Trichoderma* has the ability to parasitize healthy sclerotia

Mass production of *Trichoderma* isolates

Growth of *Trichoderma* isolates in different substrates: A more complete cover of the medium by *T. harzianum* and *T. viride* was observed in Barley and Paddy within a short period than in other substrates. Initiation of mycelium occurred early in these solid substrates. Tv1 isolate of *T. viride* grew well in Barley within 3 days. In semisolid and liquid substrates complete cover of the medium by *Trichoderma* sp occurred in 5 and 6 days respectively, due to high nutrient content. But spore yield was very low in these substrates. High nutrient content in the substrate facilitated the growth of *Trichoderma* sp. As the fungi grow faster, the nutrients become depleted from the substrate indicating the reduction of spore yield in the subsequent days.

Spore yield in solid, semi-solid and liquid substrates: Among the solid substrates Barley seeds, Paddy and Brown/White peri-carp cowpea yielded a significantly higher spore count 7 days after inoculation, while a higher spore count in maize and sorghum medium produced on or at 14 days after inoculation. However maize yielded significantly greater amount of spores than sorghum. In general, liquid and semi-solid substrates were produced poor spore load compared to solid substrates. Both liquids, semi-solid substrates produced a higher number of spores at 14 days after inoculation and afterwards the spore number declined. Rice extract was proved as good as paddy extract to produce the spores of *Trichoderma* spp. *T. harzianum* was superior in producing spores in all the liquid substrates. Both the species of *Trichoderma* were produced at or above 1×10^{10} spores g^{-1} in barley as well as well as in paddy 7 days after inoculation. However, *T. viride* Tv1 spores were significantly higher in barley and paddy. White bran and red-bran-seed-coated cowpea stands the next best substrates for the production of spores of *Trichoderma* sp. Potato dextrose semi-solid medium did not support the spore yield 14 days after inoculation and were produced as low as 1×10^{7} spores g^{-1}. Paddy and rice extract were able to produce the spores of *T. harzianum*, *T. viride* at or above 1×10^{9} spores g^{-1} at 14 days after inoculation.

The overall performance of *T. viride* Tv1 isolate is found to be superior among the isolates of *Trichoderma* with respect to inhibition of *S. rolfsii* and amenable to producing the highest spore load in solid as well as liquid substrates.

The difference of spore yield could be considered for the selection of substrate as a whole for the production of *Trichoderma*. Among the isolates tested, *T. viride* Tv1 isolates proved to be the most potent isolates among all of the substrates. Rice and paddy extraction were able to produce significant spore counts at 14 days after inoculation; they did not support a steady production of spores afterwards. Since these are considered as waste materials they can be incorporated with paddy to produce *Trichoderma* at a lower cost.

Pot trials: The *T. viride* Tv1 treated plants also harbored a vigorous root system. Though a few small brownish lesion were present on the main stem. Their frequency and severity never reached the level observed in the non-Tv1 treated plants. The ability of *T. viride* Tv1 to control *S. rolfsii* infection has been attributed to the ability of *Trichoderma* to parasitize the sclerotia (Mukherjee and Raghu, 1997). Present earlier observation on the effect of Tv1 on sclerotia of *S. rolfsii* support this attribution.

Field trial: The isolates *T. viride* Tv1 reduced the stem rot significantly compared to control. However, penetration alone doesn't lead to sclerotial degradation. And naturally produced sclerotia will have an attached soil. The environmental factors and the properties of this antagonists that lead to sclerotial attack and degradation remain to be elucidated (Henis *et al*., 1983).

Since, the collar rot pathogen is capable of rapid spread, control of the disease requires suppression of initial infection and reduction of infection rate. The soil application of the powder formulation reduces the level of the pathogen inoculum in the soil and thus the initial infections. The foliar application reduces the spread of the pathogen through leaves or the rate of infection. The *T. viride* Tv1 treatment also effectively increased the mean % of disease control (PDC) and the frequency of healthy plants (Fig. 3, Table 4). The Effect of *Trichoderma* on *Z. zamiifolia* growth was obvious; height, shoot and root weight were increased. It was

Table 4: *Z. zamiifolia* Plant growth parameters with *T. vrirde* Tv1 treatment and Un- treated control at the end of the experiment

Treatment	Plant height (cm)	Shoot weight (g)	Root weight (g)	No. of suckers
T. viride Tv1	69.80±3.2^{a}	1314.1±64.1^{a}	471.9±12.47^{a}	3.4±0.18^{a}
Un-treated control	56.28±2.9^{b}	1002.2±63.5^{b}	321.4±19.77^{b}	3.5±0.62^{a}

Data are average of five replicates in two growing seasons of the experiment. Means in a column for each treatment followed by the same superscripted letters are not significantly different according to Tukey's pair wise comparisons (p = 0.05) test

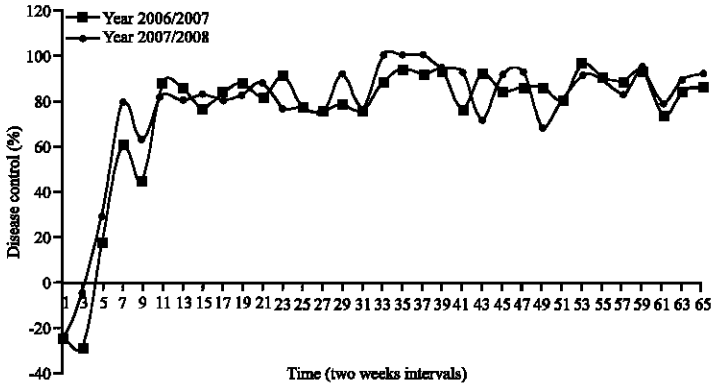

Fig. 3: Percent Collar rot Disease control in *Z. zamifolia* with *Trichoderma* treatment. PDC = $(DI_{ck}-DI_{tr})/DI_{ck}\times100$. DI_{ck}- mean disease incidence in check plot. DI_{tr}. - mean disease incidence in treated plot

observed that the *T. virirde* Tv1 treated plants growth, shoot weight and root weight were increased. The increase was significantly different from the control (Table 4). *Trichoderma* sp. is also known to provide plants with useful molecules such as glucose oxidase and growth stimulating compounds that can increase their growth and vigor (Brunner *et al*., 2005; Gravel *et al*., 2006).

CONCLUSION

Trichoderma isolates especially *T. viride* Tv1, has the potential to be used as a biological controlling agent against collar rot of *Z. zamiifolia* caused by *S. rolfsii.* Paddy or Barley based media can be used to mass produce the antagonistic fungus *Trichoderma.*

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