ANTAGONISTIC POTENTIAL OF *TRICHODERMA HARZIANUM* AGAINST *SCLEROTIUM ROLFSII* CAUSING COLLAR ROT IN BEAN

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Collar rot disease caused by *Sclerotium rolfsii* is a major problem of bean cultivation in Kandy and Matale districts under wet and humid conditions. Farmers practice seed and soil treatment with fungicides to control this disease. However, in most cases fungicides applied are not as effective as expected due to the persistence of sclerotia of this fungus in the sub-soil for many years and genetically acquired resistance of pathogen to fungicides, which continue to be the major limitations of chemical control.

Increased emphasis has been placed recently on investigating biological methods as a complete or partial alternative to chemical control of soil-borne diseases. The potential of using antagonistic *Trichoderma* spp. as bio-control agent was suggested more than 50 years ago by Weindling (1932). However, only recently antagonistic potential of *Trichoderma* spp. was exploited in plant disease control successfully in some countries.

This study was therefore, carried out to investigate the feasibility of using local isolate of antagonistic *Trichoderma* spp. as an alternative to fungicides to control *S. rolfsii* causing collar rot in bean.

MATERIALS AND METHODS

This experiment was conducted in four stages in laboratory and green house at HoRDI, Gannoruwa during *yala* 1995. Laboratory experiments focused on the isolation and bio-mass production of antagonistic *Trichoderma,* while greenhouse experiment was conducted to evaluate the efficacy of the local isolate of the *Trichoderma* sp. against *S. rolfsii* that causes collar rot disease in bean.

Fungal culture

Isolate of *S. rolfsii*, that causes collar rot in bean was used. The isolate was grown on potato dextrose agar medium.

Isolation of antagonistic Trichoderma

Soil samples were collected from Yatawatta area where collar rot disease of bean is suppressed naturally to some extend and serial dilutions were plated on potato dextrose agar medium in triplicates. The plates were incubated at 25°C for two days. A three day old mycelial preparation of *S. rolfsii* was blended for 10 seconds in 100 ml of sterilized water at low speed using a rotary mixture and sprayed on dilution plates using a sterile atomizer. The plates were incubated for additional 3-5 days. *Trichoderma* colonies exhibiting antagonistic activity were isolated, purified by repeated sub-cultures and identified.

Antagonistic property of T. harzianum

The selected isolate of *Trichoderma* sp. was grown on dual cultures with *S. rolfsii* to observe the antagonistic or hyper parasitic properties. Hyphal interactions between *S. rolfsii* and *Trichoderma* sp. were observed under the microscope. The growth inhibition and sclerotial formation of pathogen were recorded.

Bio-mass production of T. harzianum

One of the requirement for the successful bio-control is a cost effective method of producing bio-mass of antagonist (Lisansky, 1985). Agricultural waste products were used as substrates to grow the local isolate of *Trichoderma* sp. Rice hull (100 g), straw (100 g) and a mixture of rice bran : saw dust : water at the ratio of 3:1:4 were used with an addition of essential carbon, an energy source - dextrose (2%).

After 4 weeks of incubation conidia were counted using counting chamber method. The conidial suspension was mounted on haemocytometer for the counting. At least 20 squares were randomly selected and the spores were counted. Following formula was used to determine the number of spores per ml.

$$\text{Spores/ml} = \frac{\text{Dilution x total number of conidia}}{\text{Total number of squares x volume above one squares in a ml}}$$

The results are presented in table 1.

Efficacy of T. harzianum as a bio control agent

An experiment was conducted in clay pots of 15 cm x 15 cm (Diameter x height). The pathogen was grown on potato dextrose agar plates at 25°C, until the sclerotia were formed and inoculated to the moist soil at the rate of approximately 90-100 sclerotia/ 500g of soil. After 4 days cultured bio-mass of *Trichoderma* sp. was applied to the soil at the rate of 50g/ 500g of soil. Two days later seeds coated with *Trichoderma* spores and untreated seeds were sown in separate pots filled with 500g of sterilized and unsterilized soil at the rate of 3 seeds/ pot. So the experiment consisted of the following treatments and control.

Treatment	1	(T1)	-	Sterilized soil with pathogen
Treatment	2	(T2)	-	Sterilized soil with pathogen and *Trichoderma* added to the soil
Treatment	3	(T3)	-	Sterilized soil with pathogen and seed coated with *Trichoderma*.
Treatment	4	(T4)	-	Sterilized soil with pathogen and *Trichoderma* added to soil and seed coated
Treatment	5	(T5)	-	Unsterilized soil with pathogen
Treatment	6	(T6)	-	Unsterilized soil with pathogen and *Trichoderma* added to soil
Control			-	Unsterilized soil without *Trichoderma*.

Experiment was conducted in completely randomized design with 3 replicates. The seedling mortality was recorded 4 weeks after planting and the data were analyzed statistically to compare the treatments. The results are presented in table 2.

RESULTS AND DISCUSSION

Antagonistic activity of T. harzianum

The antagonistic *Trichoderma sp.* isolated was identified as *Trichoderma harzianum*. This local isolate showed a strong antagonism against *S. rolfsii* under *in-vitro* conditions. In dual culture experiment *T. harzianum* caused 71.08 % inhibition of mycelial growth and total inhibition of the formation of sclerotia of the pathogen. However *S. rolfsii* colony was completely over grown by *T. harzianum* within 6 days. The mechanism of mycoparasitism was by close contact, coiling, penetration and disintegration of the protoplasm of the pathogen.

Bio mass production

Cultured bio-mass of *T. harzianum* containing the effective conidia was produced in all three media after 4 weeks of incubation but at different concentration levels. Concentration levels of conidia per 10 g substrate after 4 weeks of incubation are given in table 1. Older cultures (15 - 25 days) were reported to have greater inhibitory effect compared to the younger cultures (Sawant and Mukhopadhyay, 1990)

Table 1: Spore density of *T. harzianum* in 10 g of substrate.

Substrate media	Mean spore density (spores/ml)
Paddy husk	2.26×10^{8}
Straw	1.73×10^{8}
Rice bran, saw dust mixture	8.91×10^{7}

Efficacy of T. harzianum in the Green house

The table 2 shows the seedling mortality rate in different treatments. The seedling mortality of 75.92 % was recorded when the sterilized soil was inoculated with the pathogen (T1). A comparison was made between the rate of seedling mortality in sterilized soil inoculated with the pathogen alone, and pathogen in combination with *T. harzianum* in the soil (T2), and in the seed (T3). The presence of *T. harzianum* in the soil and seed caused 56.21 % and 48.74 % reduction in the seedling mortality respectively. Nearly 100 % reduction in the seedling mortality was achieved when the soil and seed treatment of *T. harzianum* were combined together in a single treatment (T4).

Table 2: Antagonistic efficacy of *T. harzianum* against *S. rolfsii* measured by bean seedling mortality in pot experiment in the Green house.

Treatment	Seedling mortality (%)
T1	75.92 a
T2	19.71 de
T3	27.18 cd
T4	0.50 f
T5	50.03 b
T6	33.99 c
Control	12.48 e
C.V %	12.30

(Figures within a column followed by same letter are not significantly different at p = 0.05 % level by LSD test.)

However, in unsterilized soil antagonistic activity of the *T. harzianum* was significantly reduced probably due to the interference of other soil micro-flora. Still there was a significant reduction of the seedling mortality of 16.04 % with the addition of *Trichoderma harzianum* only to the soil (T6). The rate of seedling mortality can be further reduced, if the interference of other soil micro-flora could be minimized by adopting cultural practices that provide favourable environment for the growth and development of the introduced antagonist.

CONCLUSION

The present findings clearly show that antagonist *T. harzianum* has the potential to reduce the mortality of bean seedlings due to collar rot disease to a limited level in unsterilized soil. Despite the encouraging results of this study many serious problems and constraints exist on the road to a successful implementation of biological control programme.

However, biological control of soil borne diseases may be enhanced by adopting cultural practices such as addition of reduced amount of pesticides, solarization, nutrient and organic amendments that alter the soil environment to optimize pathogen suppression by both resident and/ or introduced antagonistic *Trichoderma* spp.

It was observed in *in-vitro* studies that the optimum pH range for the growth of antagonistic *Trichoderma* sp. was between 5.0 - 6.5. Therefore, by manipulating the pH accordingly, pathogen suppression by antagonistic *Trichoderma* sp. can be enhanced.

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