Effect of Biocontrol Agent *Trichoderma (T. viride and T. konnigii)* on Basal Rot of *Cloropytum comosum 'laxum'* Caused by *Sclerotium rolfsii*

K.A.L. PRIYADARSHANI[1], D.B.KELANIYANGODA[1] and S.SAMARAWEERA[2]

[1]*Department of Horticulture and Landscape Gardening, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila(NWP), Sri Lanka.*
[2]*Asian Cuttings Ltd, Kandawala, Katana.*

ABSTRACT

At present, the biological control of soil borne fungal diseases is becoming popular in foliage industry of Sri Lanka, which is a nature-friendly ecological approach to overcome the problems caused by standard chemical methods of plant protection. With a suitable bio control agent it can be suppressed the pathogen and reduced the disease incidence effectively. This experiment was conducted over a period of six months to identify a potential bio control agent for basal rot of *Cloropytum comosum 'laxum'* caused by *Sclerotium rolfsii* with five treatments of *Trichoderma viride, Trichoderma konnigii* and combination of *Trichoderma viride* and *Trichoderma konnigii*, Pormarsol forte 80%wp and control. The mean disease incidences of above treatments were 1.75, 2.75, 1.5, 1.75 and 10.75 respectively. It was revealed that *Trichoderma spp* are suitable for the highly effective control of plant diseases caused by *Sclerotium rolfsii.*

KEYWORDS: Biocontrol agent, Cloropytum comosum, Sclerotium rolfsii, Trichoderma

INTRODUCTION

At present, floriculture industry in Sri Lanka has shown a steady growth with a increment of export earnings from Rs. 1.68 million in 1980 to Rs. 1032 million in 2003 (Anon,2004). And in Sri Lanka around 116 nurseries were engaged in the production of export oriented floriculture products (Kelaniyangoda *et al.*, 2002).

One of the main problems of the floriculture industry in Sri Lanka is the destruction of nursery products due to plant diseases caused by phytopathogenic fungi such as *Pythium spp, Fusarium spp, Rhizoctonia* and *Sclerotium*. Among them, rots caused by soil borne fungi *Sclerotium rolfsii* is a widespread disease, which leads to greater economic loss.

Sclerotium rolfsii has an exceptionally broad host range, attacking over 200 genera of vegetables, grains, and ornamental crops (Brooke *et al.*, 2003). They attack near the base of the plant producing considerable mass of mycelium and sclerotia on the plant surface and the soil. Control of *Sclerotium* diseases is difficult as they can survive within a wide range of environmental conditions and as *sclerotia* can be disseminated by cultural practices.

Biological control of plant pathogens is an alternative to the strong dependence of modern agriculture on chemical fungicide, which causes environmental pollution and development of resistant strains (Widyastuti, 2003).

Antagonists of phytopathogenic fungi have been used to control plant diseases and 90% of such applications have been carried out with different strains of the fungus *Trichoderma* (Benitez *et al.*, 2004). The antifungal abilities of these beneficial microbes have been known since 1930s (Chet, 2006).

Success of *Trichoderma* strains as biocontrol agents is due to high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, and strong aggressiveness against phytopathogenic fungi (Benitez *et al.*, 2004). This antagonism is due to the mycoparasitism, antibiosis, and competition for nutrient and space, increase plant tolerance to the stress, induce resistance and inactivation of pathogens enzyme and solubilization and sequestration of inorganic nutrients.

Biological control of *Sclerotium rolfsii* has been subjected to many researches and number of antagonistic fungi has been shown to provide control against *Sclerotium rolfsii* in controlled experiment conditions (Stephen *et al.*, 1992).

There for this experiment was conducted to identify efficacy of bio control agent against *Sclerotium rolfsii.*

MATERIALS AND METHODS

The laboratory experiments were carried out at the faculty of agriculture and plantation management, wayamba university, Mankandura and the field experiments were carried out at Asian cuttings pvt. Ltd., Kandawala, Kandana.

A) Laboratory Experiments

Isolation of Sclerotium rolfsii

Isolation of *Sclerotium rolfsii* was done from diseased *Cloropytum* plants obtained from the Asian cuttings pvt. Ltd. Sclerotia on diseased plants were harvested and washed with 5% bleach solution and then 2-3 times with distilled water. Then the sterilized sclerotia were placed on a filter paper for blot drying and then placed in petri dishes containing Potato Dextrose Agar (PDA). The cultured petri dishes were incubated at 27°c. After 7-10 days the sclerotia were harvested and stored under 5°c in refrigerator until they are used for the inoculation.

Mass Culturing of Trichoderma

Five kilograms of paddy seeds were washed thoroughly and soaked for 24 hrs. Then they were boiled until the husk was split. 200g of parboiled rice was put in to polypropylene bags and these bags autoclaved at 121 ^{0}c for 20 minutes under 15 psi. Then two grams of glucose was added to each bags. Then *Trichoderma viride and Trihoderma konnigii* cultured on PDA were inoculated in to bags separately and incubated at room temperature for about three weeks.

Assays on Testing Antagonistic Effect of Trichoderma spp. Against Sclerotium rolfsii Under In-Vitro Conditions

The *Trichoderma viride, Trichoderma konnigii* and combination of *Trichoderma viride, Trichoderma konnigii* were cultured on PDA media separately with *sclerotium rolfsii.*

b) *Field Experiments*

Efficacy of Fungus Trichoderma against Sclerotium rolfsii under in- Vivo Conditions

500 *Clorophytum comosum' laxum'* plants were selected and planted in 10 cm diameter pots. The media was the coir dust and sand in 1:1 proportion. Experimental design was Completely Randomize Design (CRD) and consists of five treatments (Table 1). Each treatment had four replicates with 25 plants/replicate. Pots were maintained under green house conditions.

Table 1 - Details of Treatments:

Treatment	Rate of Application
1.*Tricoderma viride*	150g/m^2(>1×10^{6}sp/ml)
2.*Tricoderma konnigii*	150g/m^2(>1×10^{6}sp/ml)
3. combination of *Tricoderma viride and Tricoderma konnigii*	150g/m^2(>1×10^{6}sp/ml)
4.Pormarsol forte 80% wp	4g/5l/m^2
5.untreated control	-

Artificial inoculation of the *Sclerotium rolfsii* was done with the harvested sclerotia. After three days of the inoculation treatments were applied. *Trichoderma* grain cultures with spore concentration >1×10^{6}sp/ml were selected for field application. For treatment one to three, *Trichoderma grain* culture was incorporated into the soil after forking the soil around the root zone and for treatment four the soil was drenched with the Pormarsol forte 80%wp.

Weeks after treatments the number of plants showing symptoms was recorded. The data were recorded five days interval. Results were analyzed using ANOVA and LSD in SAS computer package.

Determination of Survival of Trichoderma Under Field Condition

Soil samples were taken from the *Trichoderma* inoculated pots at weekly intervals. Then the dilution series was made on PDA for counting colonies of *Trichoderma spp.*

RESULTS AND DISCUSSION

Assays on Testing Antagonistic Effect of Trichoderma spp. Against Sclerotium rolfsii Under In-Vitro Conditions

Under *in-vitro* conditions both *Trichoderma* spp. inhibit the mycelia growth of *Sclerotium rolfsii* and produced sclerotia. Once in contact the *Trichoderma* produced several fungi toxic cell wall degrading enzymes and probably also peptaibol antibiotics .The combined activities of these compounds result in parasitism of the target fungus and dissolution of the cell wall (Harman *et al.*, 2004). *Trichoderma* isolates differed in suppressing the *Sclerotium rolfsii.* Inhibit zone of the *Trichoderma konnigii* was 4.5cm while the inhibit zones of the *Tricoderma viride,*and the combination of *Tichoderma spp.* were 2.5cm and 2.2cm respectively (Table 2).It shows that *Trichoderma konnigii* has created inhibit zone with high diameter than other two treatments.

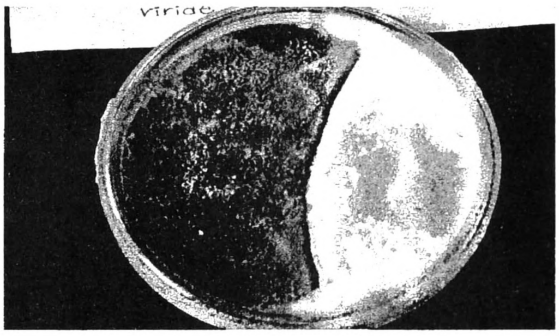

Figure 1 - Inhibition of the Growth of *Sclerotium rolfsii* by *Trichoderma viride*:

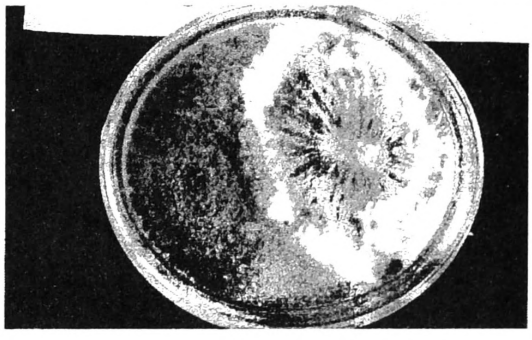

Figure 2 - Inhibition of the Growth of *Sclerotium rolfsii* by *Trichoderma konnigii*:

Efficacy of Fungus Trichoderma against Sclerotium rolfsii under Field Conditions

The mean disease incidences of T_1, T_2, T_3 and T_4 are significantly different from the control treatment (table 3). There was no significant difference among

T_1, T_2, T_3 and T_4. The mean disease incidence of treatments with *Trichoderma viride, Trichoderma konnigii*, and the combination of *Trichoderma viride* and *Trichoderma konnigii* were 1.75, 2.75 and 1.5 respectively while control showed 10.75. It is obvious that there was a significant reduction in disease incidence of treatments with *Trichoderma* compared to the control.

Table 2 - Effects of *Trichoderma* Against *Sclerotium rolfsii* Under In Vitro:

Treatment	Diameter of inhibit zone(cm)
1.*Trichoderma viride*	2.5
2.*Trichoderma konnigii*	4.5
3. Combination of *Trichoderma viride* and *Trichoderma konnigii*	2.2

Table 3 - Mean Disease Incidence Of *Sclerotium rolfsii* Under Field Conditions:

Treatment	Mean Disease Incidence
T_1 *Trichoderma viride*	1.75^b
T_2 *Trichoderma konnigii*	2.75^b
T_3 combination	1.5^b
T_4 Pormarsol forte 80%wp	1.75^b
T_5 untreated control	10.75^a
CV%	14.23
LSD (p=0.05)	2.17

Means followed by the same letter in each column are not significantly different at p=0.05 level.

This reduction may be due to the direct interaction between pathogen and *Trichoderma* as in mycoparasitism, which involves physical contact, coil around or grows along the host hyphae, recognition of the host by mycoparasite, excreation of extracellular enzymes and lysis of the host.Chet *et al.* ,(2004) reported that in *Trichoderma* this reaction has been found to be rather specific and lectin-carbohydrate interactions were assumed to meadiate the attachment and recognition between *Trichoderma* and soil borne plant pathogenic fungi. And the *Trichoderma* produces antibiotics that act as synergistically with the enzymes and inhibit the growth of other microorganisms.

Bio control of plant diseases by *Trichoderma* can be resulted by the competition for nutrients and space also. *Trichoderma* strains grow rapidly when inoculated in the soil, because they are naturally resistant to many toxic compounds, including herbicides, fungicides, and pesticides such as DDT, phenolic compound (Chet *et al.*, 1997).

In addition to the ability of *Trichoderma spp.* to directly attack or inhibit growth of plant pathogens these fungi can also induce systemic and localized resistant to a variety of plant pathogens (Chet,2004).According to the Benitez, 2004 *Trichoderma* can even exert positive effects on plant with an increase in plant growth (biofertilization). In biofertilization root colonization by *Trichoderma* strains result improvement of root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients.

Number of sclerotia produced in the pots treated with *Trichoderma* was higher than the number of sclerotia in control .This greatest sclerotial formation is due to the unfavorable conditions created by the *Trichoderma* against *Sclerotium*. But germination of those sclerotia inhibit by the *Trichoderma*.

Compared to the control the chemical treatments with Pormarsol forte 80%wp have shown better results against Sclerotium rolfsii. But it is not durable, cost effective and environmental friendly. Harman, (2004) has reported *Trichoderma* strains are widely used for disease control in green house industry instead of chemical fungicides because, it is safer to use for growers, its disease – control effects last longer than those of synthetic chemical pesticides .So, it is less costly than chemical fungicides and root growth can be as good or better than that achieved using pesticides.

In the control treatment the disease incidence is significantly higher than other treatments as the pathogen does not have any harmful or antagonistic effects from other fungi or chemical.

Survival of Trichoderma under Field Conditions

During this experiment it is observed that *Trichoderma* colany number was started to reduce after the second week of the application (Figure 3). Kelaniyangoda *et al.*, (2004) reported that two weeks after field application of *Trichoderma* colany number of the population started to reduce with the time and subsequently it went bellow the minimal effective level.

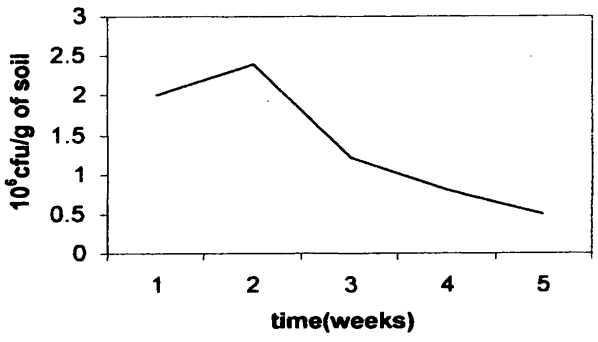

Figure 3 - Survival of *Trichoderma* In Soil:

The survival of *Trichoderma* under field conditions depends on the food base, pH, moisture and type of soil. By introducing *Trichoderma* to the soil along with a food base like rice grains, rice bran,

coconut scrapings (after milk extraction) may be more successful. *Trichoderma* develop more rapidly on acid soils than in alkaline soils and lasted longer in moist soil than in dry soil.

To obtain good results from the *Trichoderma* the spore concentration should be maintain at the optimum level .For that the repeated application of the *Trichoderma* should be practiced.

CONCLUSIONS

Trichoderma viride, Trichoderma konnigii and combination of them can be used for successful bio control of the basal rot of *Clorophytum comosum* caused by *Sclerotium rolfsii. Trichoderma* can survive 3-4 weeks within the soil. To avoid the sclerotial germination and to control the disease successfully the desired spore concentration should be maintain by the repeated application of *Trichoderma* in to the soil.

ACKNOWLEDGEMENTS

The authors wish to thanks the staff of the Asian Cuttings Ltd, Kandawala, Katana.for providing facilities to carry out the experiment. And also the authors would like to offer their sincere thanks to Prof. J. S. B. Jayasekara, Dean, Faculty of agriculture and Plantation Management, Wayamba University .Finally, the help given by Mr. Padmasiri, Technical officer and the staff of the Department of Horticulture and Landscape Gardening is greatly appreciated.

REFFERENCES

Anon (2004). Eurostat CBI reports, Colombo: Export Development Board, Sri Lanka.

Benitez T., A.M. Rincon, M. Carmen Limon, A.C. Codon.(2004). "Bio Control mechanisms of *Trichoderma* strains". International Microbiology, 7:249-260.

Brooke A. Edmunds, Mark L.Gleason(2003). "Perennation of *Sclerotium rolfsii* var. *Delphini* in lowa."Plant Health Progress.On line publication doi :10.1094/PHP-2003-1201-Rs

Chet I., J. Inbar, I. Hadar (1997). "Fungal antagonists and mycoparasites." Environmental and microbial relationships,165-184pp.

Chet I., A. Viterbo,M.Shoresh, M. Harel.(2004). "Enhancement of plant disease resistence by the bio control agent *T. asperellum*."Faculty of bio chemistry, 8,9pp.

Harmen G. E. , C.R. Howell A.Viterbo, I.Chet, M. Lorito (2004). "*Trichoderma speices* – opportunistic,avirulent plant symbionts." Nature Reviews , 2:43-56.

Kelaniyangoda,D.B., N.P.H. Nimalananda, A.C.U. Senarathne and R.S.Y.De Silva (2002) . "Identification of plant pathogens associated with foliage nurseries." In:Annals of the Sri Lanka Department of Agriculture,4:281-291.

Kelaniyangoda, D. B., S. D.Jayasekara, W. M. D. Kumari, M. H. A. D. Subashini (2003). "Effect of biocontrol agent Trichoderma viride isolates on soil borne diseases in foliage plants." In:Annals of the Sri Lanka Department of Agriculture,5:121-128.

Stephen A. Ferreira, Rebecca A. Boley (1992) "*Sclerotium rolfsii.*" Crop Knowladge master Available from: http://www.extento.hawaii.edu/Kbase/Crop/Type/s_rolfs.htm.

Widyastuti S. M., Harjono, Sumardi and D. Yuniarti (2003). "Biological Control of Sclerotium rolfsii Damping-off of Tropical Pine (Pinusmerkusii) with Three Isolates of Trichoderma spp.." OnLine Journal of Biological Sciences,3(1):95-102.