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EFFECT OF BIOCONTROL AGENT *Trichoderma viride* ISOLATES ON SOIL-BORN DISEASES IN FOLIAGE PLANTS

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ABSTRACT

Soil –borne diseases in foliage plants have become a serious problem in recent years. Biological control is possible if suitable biocontrol agent is found. A laboratory study was initiated at National Plant Quarantine Service, Katunayake over a period of six months to isolate and identify organisms antagonistic to pathogens. In an i*nvitro* study, *Trichoderma viride* was tested as a potential biocontrol agent against the fungal diseases caused by *Fusarium* sp and *Pythium* sp. The effect of this biocontrol agent was also tested in the field at Mike Flora, Rambukkana over a period of six months. Here effect of *T. viride* was compaired with other approaches such as fumigation and chemical treatment in controlling soil borne diseases in croton variety 'Gold son'. The mean percentages of disease incidence in *Trichoderma* treatment, chemical treatment and fumigation treatment were 1.67, 2.66 and 0.00 respectively, while control showed 11.99% disease incidence. The diseases caused by *Fusarium* spp. and *Pythium* spp. could be successfully controlled by using *T. viride* under field conditions compared with other methods tested.

KEYWORDS: Bio control agent, *Codiaeum*, *Fusarium, Pythium, Trichoderma viride.*

INTRODUCTION

Biological control of soil borne diseases is a popular and challenging goal, which has been a subject of research for many years. It is attractive in environmental and economic sense because it offers durable, safe and cost effective alternatives to soil applied chemicals (Hornby, 1990). A microbial antagonist can efficiently suppresses pathogens and reduces disease incidences. Antagonism may be by competition, antibiosis and/ or exploitation and the best biocontrol agents may suppress pathogens by a combination of these methods (Chet, 1987). Parasitism by a biocontrol agent as a mechanism of disease control is an attractive concept because it could reduce inoculum density. The basic idea in biological control is to utilize a fungal or bacteria colonizer that would antagonize pathogens at the root infection site, which would result in reduced infection (Kelaniyangoda, 1992).

Genus *Trichoderma* represents widely studied fungi that show antagonistic activity towards soil-borne pathogens. Weindling and Fawcett (1936), first demonstrated the parasitic activity of members of the genus

Trichoderma to pathogens such as *Rhizoctonia solani***.** Later other mycoparasites such as *Coniothyrium minitans, Tilletiopsis* sp., *Sporidesmium sclerotivorum* and *Pythium nunn* were tested as biocontrol agents against target soil borne plant pathogenic fungi (Hornby, 1990). *Trichoderma*, was shown to actively invade the mycelia of *R. solani* and *Pythium* sp. and to produce the lytic extra cellular enzymes chitinase and ß–1,3–glucanase *in vitro* (Chet and Baker, 1980).

The objective of the experiments reported here were to isolate potential biocontrol agent, *T. viridae* in the laboratory and test its effectiveness for control of soil born diseases of croton (*Codiaeum*) in the field.

MATERIALS AND METHODS

The laboratory experiments were conducted in the Plant Pathology Laboratory of the National Plant Quarantine Service at Katunayaka. Field experiments were conducted at the Mike Flora nursery at Rambukkana.

Laboratory Investigations

Isolation of causal organisms

Isolation of causal organisms was done from diseased *Codiaeum* plants obtained from the Mike Flora nursery. Root segments from diseased *Codiaeum* plants var. 'Gold Sun' were thoroughly washed in running tap water for 2-3 times until soil was completely removed. Thoroughly washed pieces of roots were placed on a filter paper for blot drying and then placed in petri dishes containing water agar. The petri dishes were observed under microscope for fungal growth, after incubating at 25^0 C (48 h)

Fungal isolates were transferred to Corn Meal Agar (CMA) for further identification. The presence of fungal pathogens other than *Pythium* sp. on surface sterilized diseased root segments cultured on potato dextrose agar (PDA). The fungi growing on the medium were isolated and identified using morphological characters. Pure cultures were maintained on PDA by regular sub culturing.

Isolation of *Trichoderma*

The isolates of *Trichoderma viride* were obtained from National Plant Quarantine Service (NPQS) culture collection. They were inoculated onto PDA and incubated at room temperature (28º C) for 7 days. The fungi growing on the medium were isolated and identified using morphological characters including type and arrangement of the spores (Rifai, 1969).

Trichoderma as an antagonistic fungus against *Fusarium* and *Pythium* under *in vitro* conditions

The *Trichoderma* isolate was inoculated on PDA medium with *Fusarium* sp., and on CMA medium with *Pythium* sp. to test antagonistic reactions.

Mass culturing of *Trichoderma*

Two kilograms of paddy seeds were washed thoroughly and soaked for 24 hours and boiled until husk was split. They were sieved, and about 200g of parboiled rice was put into polypropylene bags and 2g of glucose was added to each bag. These bags were autoclaved at 121º C for 20 minutes, under 15 psi. *Trichoderma* sp. cultured on PDA was inoculated into each bag and incubated at room temperature (28º C) for about 3 weeks until well sporulated.

Field Experiments

Effect *T. viride* for control of *Fusarium* and *Pythium* spp. in *Codiaeum* sp. under field conditions

Naturally infested field plots with one-year-old *Codiaeum* varieties 'Gold sun' and 'Gold star' were selected for the experiment. Experimental design was randomized complete block and consisted of 4 treatments (table 1). Each treatment had 4 replicates with 75 plants/plot.

Table 1. Treatment combinations.

Treatments	*Rate of application*
1. *Trichoderma*	150g/m^2 (>1x10^6 sp/ml)
2. Pormarsol forte 80% (Thiram 80%)	4g/5l/m^2
3. Fumigant (Bazomid)	20 g/m^2
4. Untreated control	--_

Grain cultures with spore concentration > 1 x 10^6 spores/ml were selected for field application. For treatment 1 experimental plots, *Trichoderma* rice grain culture was incorporated into the soil after forking the soil around the root zone and for treatment 2 experimental plots, the soil was drenched with chemical at one week intervals. Four weeks after treatments, the number of plants showing symptoms was recorded. Results were analyzed using ANOVA and LSD in SAS package.

Determination of survival of *Trichoderama* under field conditions

The survival of *Trichoderma* sp.in soil was assessed by a serial dilution technique. Soil samples (10g) were taken at weekly intervals from *Trichoderma* inoculated fields, and a dilution series was made on a PDA medium, for counting colonies of *Trichoderma* sp.

RESULTS AND DISCUSSION

Identification of causal organisms

Both *P aphanidermatum* and *P. splendens* are known to cause root rot in *Codiaeum* spp. (David *et al.*, 1989). Culturing of root segments on corn meal agar yielded both *Pythium* and *Fusarium* sp. It appears that a combination of *Pythium* sp. and *Fusarium* sp. may cause root rot in tested *Codiaeum* sp. However, *Pythium* sp. rarely attack mature plant root system. Infection by *Fusarium* sp. may be supportive of *Pythium* ultimately leading to pathogen interaction and rotting of roots.

Antagonism of isolates of *T. viride* against *Fusarium* and *Pythium* spp. under *in vitro* conditions

The isolates of *Trichoderma viride* inhibited myciliam growth of *Fusarium* sp. on potato dextrose agar sometimes showing over growth. The lytic enzymes secreted by *Trichoderma* can degrade the cell walls of *Fusarium.* Due to bacterial contaminations of cultures, clear antagonistic effect of *Trichoderma* sp. on *Pythium* sp. was not observed. However, reports indicate that *Trichoderma* produces ß –1-3-glucanase, chitinase and cellulase that are potentially capable of degrading the cell walls of *Pythium* spp. and *R. solani* (Elad *et al*., 1983).

Effects *T. viride* against *Fusarium* and *Pythium* spp. in under field conditions

The mean disease incidence under different treatments is given in table 2. Treatments T1, T2 and T3 are significantly different from the control treatment. However, there was no significant difference among T1, T2 and T3. When *Trichoederma* rice grain culture alone added to the infested field (T1), there was a significant (p = 5%) reduction in disease incidence compared to the control.

Table 2. Combined mean disease incidence of *Pythium/ Fusarium* under field conditions

Treatments	*Mean Disease Incidence (%)*
T1-*Trichoderma*	1.66b
T2-Thiram 80%	2.66b
T3-Bazomid/Fumigant	0.00b
T4-Untreated control	11.99a
CV%	19.5
LSD (P=0.05)	4.33

Means followed by the same letter in each column are not significantly different at p=5% level

This reduction was most probably due to the mycoparasitic activity of *Trichoderma* spp. on soil borne pathogens such as *Fusarium* sp. *and Pythium* sp. Mycoparasitic process apparently includes chemotropic growth of *Trichoderma*, recognition of the host by the mycoparasite, excretion of extra-cellular enzymes and lysis of the host Elad *et al.* (1983) reported that in mycoparasitism, the parasite sometimes penetrates the host mycelium by partially degrading the cell wall. A classic paper by Weindling (1931-1941) reported that, *T. viride* is one of the antagonists to soil borne pathogens. Also, it is an active antagonist in moist soil under very wet conditions when the soil pH was lower than 6.5. Since pH of experimental plots was around 5-6, this condition is somewhat favorable for *Trichoderma* growth. Weindling and Fawcett (1936) reported that, without acidification of the soil, introduced *T.viride* was ineffective as a biological control agent.

In addition, to the mycoparasitic activity, *Trichoderma* spp may also compete with plant pathogens for nutrients and other requirements. This may also be a cause for reduced disease incidence in *Trichoderma* treated plots compared to the control. Even though chemical treatment lowered the disease incidence considerably, it could not completely prevent it. Chemical control for soil borne diseases is not always practicable because any application as a drench to the soil may not reach the target pathogen. In addition, some pathogens are resistant to some chemicals. Therefore, specific chemicals should be selected for each pathogen. Most chemical compounds are highly degradable. Therefore frequent application is needed to achieve the desired disease control. Pormarsol forte 80% W.P. (Thiram) is a widely used fungicide that controls root rot diseases caused by *Pythium* spp. or *Fusarium* spp. It is widely used as a seed treatment.

In this experiment No disease incidence was recorded in fumigated soils. For soil fumigation Bazomid (Dazomet) was used. Chemical fumigation usually leaves only fumigant tolerant micriflora, particularly at the edges of the treated soil beds. With the fumigation, all the pathogenic fungi got destroyed. Therefore, after fumigation of infested soil, disease incidence was reduced or absent for sometime. But after soil fumigation, soil is susceptible to

re-infestation by incoming pathogens (Roger and Yepsen, 1976). According to Roger and Yepsen, (1976), when fumigants are applied to the soil, several deleterious effects take place. For instance, the population of some soil organisms particularly fungi are lowered, while bacterial groups less affected by the chemicals multiply far beyond those in untreated soil because their antagonists and competitors are killed. This is one major disadvantage of fumigation.

A higher percentage of disease incidence, was observed in the control, experiment compared to the other treatments. Since field plots are naturally infested with *Pythium* and *Fusarium* spp., no uniform disease incidence was observed in each replicate. If the environmental conditions are favorable for disease development, disease severity is higher during the rainy season. However, within the experimental period, climatic conditions were somewhat drier and higher temperatures were experienced. Fumigation could be a very good treatment in controlling soil borne pathogens for a short period. However the possibility of re-infestation of pathogens is high when compared with the other treatments. Also, fumigation by chemicals involves a high cost and it is not environmental friendly. Use of bio control agents such as *Tricoderma* spp. is much effective, because the cost is less for application. Its persistence in the environment can be maintained at required level by frequent application. Therefore the effect of bio control agents can be continued for a prolonged time period.

By making the soil environment favorable for *Trichoderma* growth through provision of favorable pH conditions, it is possible to obtain a higher effectiveness. An antagonist introduced along with a food base may be more successful in establishing itself in soil. Use of rice grain culture as a medium for mass culturing of *Trichoderma* has given satisfactory spore concentration levels. An alternative such as locally available agricultural by- products like, rice husk, rice bran, straw could also be used (Chet, 1990). The advantage of introducing a substrate with the biocontrol agent was apparent and the persistence of *Trichoderma* added with the wheat bran mixture gave better-extended control when the agent was only in the form of conidia. *Trichoderma* can be applied together with various soil treatments for long-term control of soil borne pathogens. Furthermore recent findings show that *Trichoderma* directly affects plants and can live in their roots (Mukerji and Garg, 1993). It enhances plant growth and flower production and may serve as a growth promoter.

Survival of *Trichoderma* under field conditions

Chet and Baker (1980) found that the minimal effective amount of *Trichoderma* is about 10^{6}cfu/g of soil. This level can be kept in the soil for several months depending on the food base applied with the agent, type of

soil, pH, temperature, and moisture conditions. In moist soil, *Trichoderma* survived longer than in dry soil (Mukerji and Garg, 1993). Figure 1 shows second week after inoculation colony numbers of the population of *Trichoderma* started to reduce with the time. Subsequently it went below the minimal effective level. Therefore, repeated application should be done in order to maintain a good spore concentration.

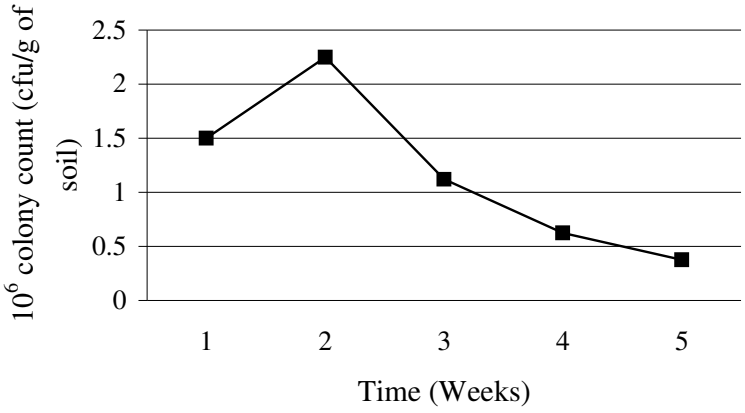

Figure 1. Survival of *Trichoderma* in soil.

CONCLUSIONS

Root rot disease of *Codiaeum* spp. was mainly caused by *Pythium* sp., while *Fusarium* spp. acted as an associated pathogen. The two pathogens can be effectively controlled by *Tricoderma viride.* It can be more cost effective and environmental friendly than chemical treatments. The survival of *Trichoderma* spp. under normal field condition is about 4-5 weeks. Therefore, repeated application should be practiced to maintain the desired population.

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