Asian Journal of Plant Pathology

ISSN 1819-1541

Academic Journals Inc.

www.academicjournals.com

Asian Journal of Plant Pathology 3 (3): 50-60, 2009
ISSN 1819-1541

Control of *Fusarium oxysporum* Wilts Disease of *Crossandra infundibuliformis* var. Danica by *Trichoderma viride* and *Trichoderma harzianum*

[1]V. Jegathambigai, [2]R.S.W. Wijeratnam and [3]R.L.C. Wijesundera
[1]Green Farms Ltd., Marawila, Sri Lanka
[2]Research and Development Unit, Industrial Technology Institute,
363, Bauddhaloka Mawatha, Colombo 07, Sri Lanka
[3]Department of Plant Science, University of Colombo, Sri Lanka

Abstract: *Crossandra infundibuliformis* var. Danica is a very valuable ornamental flowering potted plant that has been introduced to the international floriculture market. A wilt caused by *Fusarium* oxysporum is a major problem in the production of *C. infundibuliformis* plants. Control of *F. oxysporum* causing wilt disease of *C. infundibuliformis* var. Danica was investigated using *Trichoderma* isolates. The isolates effected the growth of *Fusarium* in laboratory experiments. The results suggest that the effect on fungicidal. The *Trichoderma* isolates also effectively reduced the wilt incidence in field experiments. Further, the isolation promoted the growth of the plants. The study strongly suggests that *Trichoderma* isolates, especially *T. viride* Tv1 can be exploited for the biological control of wilt disease at field level.

Key words: *Trichoderma viride*, *Trichoderma harzianum*, biological control, field trial, *Fusarium oxysporum*

INTRODUCTION

Crossandra infundibuliformis (Fire cracker), which is widely used as a potted flowering, landscape plant in Sri Lanka and also has a great demand from many European and Asian countries. Sri Lanka, exports more than 600000 *C. infundibuliformis* rooted cuttings per annum. One of the major limiting factors of *C. infundibuliformis* monoculture is wilt caused by *F. oxysporum*. This is one of the most difficult pathogen to control and it causes destruction of the entire plant. The disease can affect the crop at any stage of growth. Characteristic symptoms are sudden drooping of leaves and petioles, no external rotting of roots, black internal discoloration involving xylem and pith. The control of *F. oxysporum* on *C. infundibuliformis* is difficult to achieve because the long survival of the pathogen in the soil as chlamydospores or as a mycelium in infected plant debris. The soil borne antagonistic fungi, *T. viride* and *T. harzianum* have been identified as naturally existing potential biological agent against *F. oxysporum* (Dubey *et al*., 2007; Shanmugam *et al*., 2008; García *et al*., 1997). It has been suggested that *Trichoderma* sp. isolated from the root or rhizosphere of a specific crop may be better adapted to that crop and be more effective in controlling *Fusarium* sp. (Rubio-Perez *et al*., 2008). The pathogen is soil borne and hence chemical control is uneconomical and cause environment and groundwater pollution. In the

Corresponding Author: V. Jegathambigai, Green Farms Ltd., Marawila, Sri Lanka

recent years due to the development of resistance in the pathogen, the usage of chemical fungicides proved to be less effective in the control of *Fusarium* sp. (Reid *et al*., 2002). Further it is also reported that treatments with fungicides have been ineffective in controlling *Fusarium* sp. under conducive condition for disease development (Federico *et al*., 2007). Hence, the need has arisen to develop alternative methods to control the disease and one such promising method is the use of the antagonistic fungus *Trichoderma* (Gilardi *et al*., 2008). *Trichoderma* sp. are well documented as effective biological control agents of plant disease caused by both soil-borne fungi and leaf-and fruit-infecting plant pathogenic fungi. These mycoparasites not only have direct effect such as protection against attack by different plant pathogens in the rhizosphere, but also have a stimulating influence on the plant growth and induction of defense responses (Ilan *et al*., 2009). Root penetration is achieved by secretion of cellulolytic and proteolytic enzymes (Ilan *et al*., 2009). *Trichoderma* species are plant symbiont opportunistic virulent organisms, able to colonize plant root by mechanisms similar to those of mycorrhizal fungi. Root colonization by *Trichoderma* species frequently enhances root growth and development, crop productivity, resistance to abiotic stress and uptake and use of nutrients (Mukhopadhyay, 2009). Root-fungus association stimulates plant defense mechanism (Mukhopadhyay, 2009).

In this investigation, four local isolates belonging to two species of *Trichoderma* (*T. viride* and *T. harzianum*) were evaluated against *F. oxysporum*.

MATERIALS AND METHODS

This experiment was carried out in Green Farms Limited, Marawila, situated in low county intermediate zones of Sri Lanka. Climatic conditions of this research site are, average annual rain fall (in 30 years)-1620 mm, minimum and maximum relative humidity 60-90%, minimum and maximum day temperature 25-34°C, minimum and maximum night temperature 20-27°C. In this study, potential *Trichoderma* sp. isolated from Marawila, Sri Lanka were tested for *in vitro* study and in field experiment antagonistic activities on the development of vascular wilt disease on *C. infundibuliformis*. Five strains of *Trichoderma*, originally isolated and identified from ornamental foliage cropped soil of Green Farms Ltd., Marawila, Sri Lanka and were identified as *T. harzianum* and *T. viride*. A strain of *F. oxysporum* originally isolated from naturally infected *C. infundibuliformis*, was used in *in vitro* study. These isolates were kept in 15% glycerol and frozen at -4°C. During the growing seasons of the years 2007/2008 (January 2007 to August 2008) the potential of different strains of *T. harzianum* and *T. viride* were evaluated to control *F. oxysporum* and the effect on plant growth promoter on *C. infundibuliformis* were determined.

In vitro Evaluation of *Trichoderma* Against *F. oxysporum*

Isolation of Microorganisms

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. *Trichoderma* species was isolated from organic rich soil within a 15 cm depth by plating soil suspension after necessary serial dilution directly on PDA. Five millliliter of soil suspension was placed in 15 mL molten, cooling PDA, swirled and allowed to solidify. The set up was incubated 5-7 days at 28°C. The isolates were purified by the single spore method. The fungi were identified on the basis of their morphological and reproductive characters (Anonymous, 2006; Bisset, 1991; Lieckfeldt *et al*., 1999; Samuels *et al*., 1998; Watanabe, 2002a) and the pure cultures of *Trichoderma* were maintained on PDA medium and stored at 4°C.

The following isolates were obtained, *T. viride*1 Tv1, *T. viride*2 Tv2, *T. viride*3 Tv3, *T. harzianum*1 Th1 and *T. harzianum*2 Th2.

Isolation of *Fusarium oxysporum*

Isolation from Plant Samples

Fusarium oxysporum was isolated from naturally infected *C. infundibuliformis* var. Danica plants in the field plantation/Green Farms Ltd., Marawila, Sri Lanka. Infected *C. infundibuliformis* plants were uprooted and collected from a plantation. The samples were put in poly bags and transferred to the laboratory and kept in moist polythene bags to enhance fungal growth. The plant material (stem and root) were cut into 5 mm pieces and surface sterilized with 0.5% sodium hypochlorite solution for 5 min and rinsed trice with sterilized water. The pieces were dried with sterile filter paper and plated (3 pieces per plate) on fresh Potato Dextrose Agar (PDA) medium impregnated with streptomycin (0.5 mg mL^{-1}) and incubated for 7-10 days at 28 to 30°C. The resulting *F. oxysporum* colonies were sub cultured by transferring small mycelia plugs from the colony margins. Pure culture was obtained by sub-culturing three times.

Isolation from Soil Sample

One gram of soil from vicinity of the roots of infected plants was collected and the dilution plate technique was used to prepare soil suspensions of different dilutions. One milliliter of each soil suspension was uniformly spread over PDA. The resulting *F. oxysporum* colonies were sub cultured from PDA plates by transferring small mycelia plugs from the colony margins. Pure culture was obtained by sub-culturing three times. The fungus was identified based on morphology and colony characteristics (Watanabe, 2002b). The pathogenicity of the isolates was established by following the Koch's postulates (Riley *et al*., 2002).

Preparation of Conidia Suspensions

Conidia suspension of the isolates of *Trichoderma* and *F.oxysporum* were prepared from 7 days old cultures grown 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.

Antagonistic Effect of *Trichoderma* sp.

Dual Culture Technique

The isolates of *T. harzianum* and *T. viride* were screened individually against *F. oxysporum* through the dual culture technique (Singh *et al*., 2004). Complete randomized design was used with five replicate and the same trial was repeated three times. Each replicates has three plates. The test *Trichoderma* isolates and the *F. oxysporum* isolate was inoculated at the center of two parallel radial lines on 9 cm diameter PDA plate. The fungi for inoculations were obtained from the margins of actively growing 7 day old cultures on PDA. The dual cultures were incubated at 28-30°C for 10 days and measurement of radial mycelia growth of the *F. oxysporum* were taken 3 and 7 days after inoculation. *Fusarium oxysporum* alone was maintained as the control (Singh *et al*., 2004). The percentage growth inhibition (I) was calculated using the formula given below (Datta *et al*., 2004):

$$[I\% = (C-T)/C] \times 100$$

Where:

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

Interaction Between Conidia of *Trichoderma* Isolates and *F. oxysporum*

One milliliter (1×10^4 conidia mL^{-1}) of the conidia suspension of the test *Trichoderma* isolate and *F. oxysporum* was introduced in to sterile Petri dishes and thereafter 15 mL molten agar (30°C) was poured. The plates were swirled round in a clockwise direction to ensure mixing of the two suspensions and the plates were incubated at 28-30°C. After 10 days inoculation the plates were observed for the presence of any pathogen colonies.

Field Experiments

An experiment was carried out under field condition in the growing seasons 2007 and 2008 in soil naturally infested with *F. oxysporum* in a farm located in the low country intermediate zone of Sri Lanka. Five treatments involving soil and foliar applications of *Trichoderma* isolates were used together with an untreated control. *C. infundibuliformis* was in field plots having 36 plants m^{-2}. The size of a replicate plot was 3×10 m. Treatments involving applications of Tv1, Tv2, Tv3 and Th1 isolates and the untreated control. Treatments were replicated three times. They were in randomized completely block designs. Standard agronomic practices were followed throughout the research period. This experiments were carried out in two growing season. The data was subjected to analysis of variance (ANOVA).

***Trichoderma* Biomass and Formulation Production**

Preparation of Solid Media

Paddy soaked in water for 6 h was parboiled in a pressure cooker (1.1 kg cm^{-2} pressure for 45 min). After parboiling the closed container was kept in a cooler room (15±2°C) for 2 h and 5 kg of 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. Plugs of uniform size of (4 mm) were obtained from a pure culture of a 7 day old *Trichoderma* isolate on PDA and used to inoculate the above media.

***Trichoderma* Liquid Formulation**

One kilogram of 7 day old mass cultures was flooded with 2 L of tap water and was shaken well in a closed container. The resulting suspension was filtered through muslin cloth. The filtrate was diluted with tap water to obtain a conidia concentration range of 10^{11} cfu mL^{-1} for field application. One liter of this conidia suspension was mixed with 1 mL surfactant (wetting agent-commercial product from Lankem Ltd.) before applications.

Evaluation of *Trichoderma* sp. Against Wilt

To perform the experiment, a field was selected where *C. infundibuliformis* var. Danica crop has been regularly grown and the soil was already infested with. *F. oxysporum*, 6 week old *C. infundibuliformis* rooted cuttings were raised on compost with dried cow dung mixed coir media. Three replication of each treatment were laid out in a randomized complete block design and 15 plots each 3×10 m^2 in size were used. Four *Trichoderma* isolates which were

found to be potent against the test pathogen during *in vitro* tests were used in the field experiments and the liquid formulation as the conidial suspension (1×10^{11} cfu mL^{-1} mixed with 0.1% surfactant) prepared as described above was used to inoculate the plants and adjacent soil. One square meter area was inoculated with 5 L of the *Trichoderma* liquid formulation and the plots were left for 14 days with sufficient soil moisture. The untreated control was drenched with water. Applications of liquid formulation were done at 2-4 weeks intervals. Infected/wilted plants were removed from the plots regularly and disease incidence/severity, percentage of disease control was assessed as below.

Measurement of Disease Incidence/Severity

Wilt incidence was recorded at periodical (2 weeks) intervals up to end of the experiment. Total wilted plants per plot were recorded. In each treatment disease development was measured by means of percent infected plants per each replicate. Rain fall, relative humidity and soil PH/EC were also measured through out the study period. The level of disease severity is expressed as the mean value of each treatment. Percentage Disease Control (PDC) was calculated by using the following equation described by Engelhard (1997) and Singh *et al*. (2002).

$$PDC = (DI_{ck}-DI_{tr})/DI_{ck}\times100$$

Where:
DI_{ck} = Disease incidence in check plot
DI_{tr} = 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 0 treated plot had the same level of infection as the check plot.

Effect of *Trichoderma* Treatments on Growth of Plants

The effect of the treatments on the growth of the *C. infundibuliformis* var. Danica was determined by measuring the growth parameters plant height, root length and fresh weight of the plant at the end of the experimental periods. Fifteen samples were randomly taken from different treatments of each replicates separately.

Experimental Design and Data Analysis

In vitro experiments were arranged as a complete randomized design with five replicates. Each replicate has three plates. Field experiments were established as a randomized complete block design with three 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) (Federico *et al*., 2007; Larry, 1997).

RESULTS

When both the species of *Trichoderma* sp. were evaluated against *F. oxysporum*, *T. viride* and *T. harzianum* were suppressed the growth of the mycelia of *F. oxysporum.*

In vitro experiments, the best four isolates each in two above species were selected based on percentage of growth inhibition and have been tested for confirmation.

In dual culture technique three days after inoculation, the mycelium of both the cultures came in contact with each other. Three days after inoculation the hyphal growth of *F. oxysporum* was found to be inhibited by the hyphae of *Trichoderma* sp. Further, *Trichoderma* almost inhibited the mycelia growth of the *F. oxysporum* at 10 days after inoculation. The advancing hyphae of *Trichoderma* covered the entire Petri plates, suppressing the growth of *F. oxysporum*. Microscopic observations in dual culture revealed that coiling of antagonistic hyphae around *F. oxysporum* hyphae that ultimately resulted in a mycelial rope like appearance. Significant differences ($p<0.05$) have been found in each treatment. Ten days after inoculation growth of *F. oxysporum* was completely restricted whereas antagonist had proliferated growth with abundant sporulation. Its mycelia strands coiled around the hyphae of *F. oxysporum* forming a rope like structure and finally disintegrated. This study clearly demonstrated that the potential of *Trichoderma* sp. as a bio control agent against *F. oxysporum* causing wilt disease in *C. infundibuliformis.*

In vitro examination of *Trichoderma* on *F. oxysporum* of *C. infundibuliformis* revealed that the *F. oxysporum* covered completely (4.5 cm) in the absence of antagonist fungus. *T. viride* 1, 2 and 3 reduced the growth of *F.oxysporum* by 93, 90.3 and 66.76%, respectively whereas *T. harzianum* 1 and 2 reduced it by 88.9 and 76.9%, respectively 7 days after inoculation.

In vitro (Hyphal Interaction)

The *in vitro* testes in dual cultures showed that all isolates of *Trichoderma* had the ability to inhibit the growth of the *F. oxysporum*. The highest inhibition was by Tv1, Tv2; Th1. Inhibition by Tv3 was lowest. The analysis of variance showed that there were significant differences between treatments and the control (Fig. 1). Macroscopic observation of fungal growth in dual culture revealed that growth inhibition occurred soon after contact between *Trichoderma* and *F. oxysporum*.

Interaction Between Conidia of *Trichoderma* Isolates and *F. oxysporum*

The entire plates were covered exclusively by the *Trichoderma* isolate (Tv1, Tv2, Tv3, Th1 and Th2) 10 days after inoculation. It was not possible to isolate *F. oxysporum* from the inoculated plates.

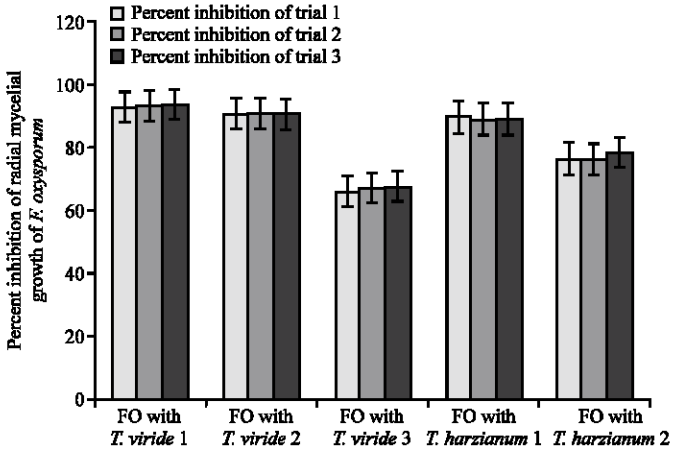

Fig. 1: Growth inhibition (%) of *F. oxysporum* by *Trichoderma* isolates. Th1: *T. harzianum1*, Th2: *T. harzianum* 2, Tv1: *T. viride*1, Tv2: *T. viride* 2, Tv3: *T. viride*3, FO-*Fusarium oxysporum*

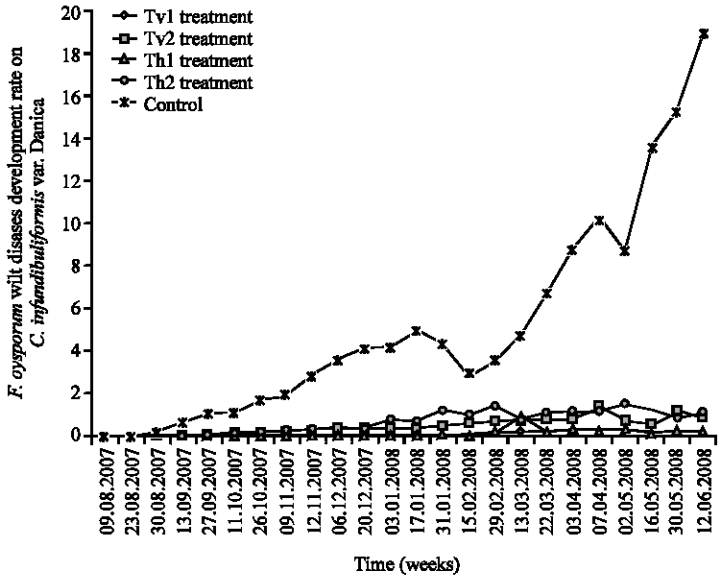

Fig. 2: Mean disease severity of *F. oxysporum* wilt disease with Tv1, Tv2, Tv3, Th1 and control treatments in year 2007/2008. Disease severity represents the percentage of the total number of plants that contained diseased plants per replicates at 2 weeks interval. Data are means of 5 replicates at 2 weeks intervals

Table 1: The effect of *Trichoderma* treatments on wilt disease severity, wilt disease reduction and growth of *Crossandra infundibuliformis* var. Danica plants

Treatments	*F. oxysporum* wilt disease incidence	Disease control (%)	Root length (cm)	Shoot length (cm)	Plant fresh weight (g)
T. viride Tv1	0.87^{a}	90.51^{a}	53.40^{a}	121.87^{a}	334.73^{a}
T. viride Tv2	4.81^{a}	82.30^{a}	54.46^{a}	113.67^{b}	330.93^{a}
T. harzianum Th1	1.38^{a}	89.16^{a}	57.00^{a}	107.00^{c}	326.73^{a}
T. harzianum Th2	6.85^{a}	77.71^{a}	54.00^{a}	105.00^{c}	311.47^{b}
Un-treated control	51.57^{b}	00.00^{b}	22.80^{b}	58.73^{d}	212.47^{c}

Means in a column for each treatment followed by the same letter(s) are not significantly different according to Tukey's pair wise comparisons ($p \leq 0.05$) test. Disease incidence presented above is the average values of occurrence of the disease at two week intervals for one year obtained from all record combined. Data are average of three replicates in two growing seasons of the experiment

Field Experiments

Biological control has the potential role in the management of diseases. *Trichoderma* species has been identified as naturally existing potential biological agent against diseases caused by fungi. Isolation of different species of *Trichoderma* sp. from the soils of foliage nurseries had been an evident to its potential role against fungal pathogens.

In field experiment 4 isolates Tv1, Tv2, Tv3 and Th1 were used. *Trichoderma* treatments were most effective in increasing the percentage of disease control (PDC) and the frequency of healthy plants (Singh *et al*., 2002). All the *Trichoderma* treatments decreased disease incidence, with TV1 having the highest disease reduction (90.51%) followed by Th1 (89.16%), Tv2 (82.30%) and Th2 (77.71%) (Table 1 and Fig. 2, 3). The *Trichoderma* treatment was enhanced plant growth leading to a significant increase in plant height, weight as related to untreated control (Table 1).

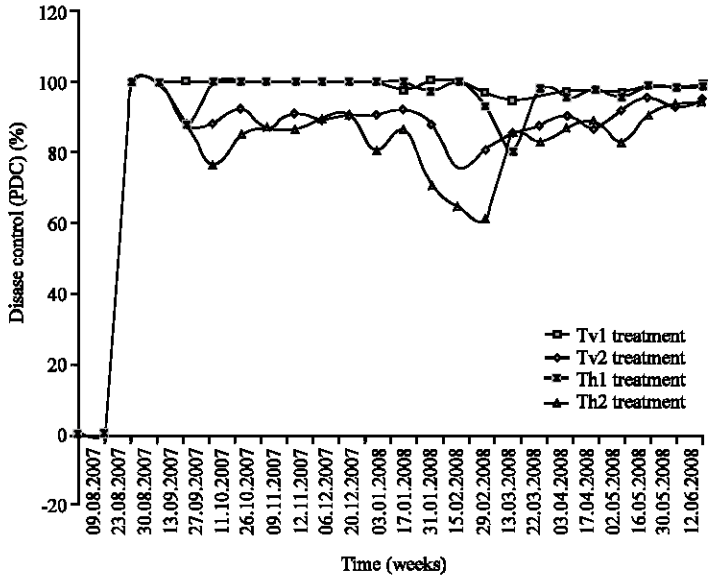

Fig. 3: Percent *F. oxysporum* wilt disease control in *C. infundibuliformis* with *Trichoderma* treatments. PDC = (DI_{ck}-DI_{tr}) / DI_{ck} ×100. DI_{ck} mean disease incidence in control plot DI_{tr} mean disease incidence in treated plot

DISCUSSION

In vitro (Hyphal Interaction)

This observation indicates that parasitization of *Fusarium* by *Trichoderma* play a role in the inhibition of *Fusarium.* Such parasitation through formation of mycelial coil has been reported by several workers (Dubey *et al*., 2007; Almeida *et al*., 2007; Irfan and Khalid, 2007; Ozbay and Newman, 2004). Production of chitinases may have direct significance in the parasitism of *Trichoderma* on *F. oxysporum* as these enzymes function by breaking down the polysaccharides, chitin and β-glucan that are responsible for the rigidity of fungal cell walls thereby destroying cell wall integrity (Howell, 2003).

Interaction Between Conidia of *Trichoderma* Isolates and *F. oxysporum*

This suggests that the effect of *Trichoderma* isolates on *Fusarium* is most likely fungicidal. Bashar and Rai (1994) reported that *T. viride* amended in soil suppressed the growth of *F. oxysporum* and exhibited strong fungistatic activity against germination of conidia of test pathogen.

Field Trial

During 2007/2008, Assessment of *F. oxysporum* vascular wilt development rate was done year around and the effect of *Trichoderma* species alone and with the control application. Results of the field experiment conducted in the year 2007/2008 were shown in Fig. 2 and 3. But *Trichoderma* alone was excelled in suppressing the wilt development and the rate was kept checked (Fig. 3). Application of spore suspensions responded positively and had ever high populations of *Trichoderma* sp. (9.2×10^{6} cfu g^{-1}) in the growing medium.

During 2007/2008, the vascular wilt disease development rate of *F.oxysporum* was kept checked after the application of *Trichoderma* alone however the control treatment were found continuously increasing the disease incidence (Fig. 2). There was no rigid correlation established between the *Trichoderma* isolates and the wilt disease development of *F. oxysporum.*

The vascular wilt development was faster in control treated plants than *Trichoderma* sp. treated plants. Since the wilt pathogen, *F. oxysporum* is capable to spread readily in *C. infundibuliformis* crop, controlling the disease requires both suppression of initial plant infections and reduction of infection rates. Applications of *Trichoderma* sp. were significantly inhibited disease severity during the initial stages of the disease development, most likely by reducing the inoculums level of *F. oxysporum* into the soil. To be successful in its performances, the bio control agent must be multiplied to sustain its effectiveness. Such effective antagonists must be established in *C. infundibuliformis* planting medium and remain active against target pathogens during periods favorable for *F. oxysporum* infections. *Trichoderma* applications enhanced the overall quality of *C. infundibuliformis* plants related to with control.

Thus, it could be concluded that use of *Trichoderma* sp. as bio-agent not only reduced the incidence of *F. oxysporum* fungi but also increased the growth and vigor of the *C. infundibulifomis*.

Repeated field investigations confirmed that *Trichoderma* sp. was a potential candidate to control wilt disease in *C. infundibuliformis*. *Trichoderma* sp. can, therefore, be used as a vital bio-controlling agent in developing effective disease management practices to manage *F. oxysporum* infections in *C. infundibuliformis* plantation (Gilardi *et al*., 2008). *Trichoderma* sp. provide plants with useful molecules such as glucose oxidase, that can increase their resistance to pathogens such as induce systemic resistance in plants (Brunner *et al*., 2005). Moreover, these fungi produce antibiotics such as gliotoxin, viridin and cell wall degrading enzymes and biologically active heat-stable metabolites such as ethyl acetate (Mujeebur *et al*., 2004). These substances may be involved in disease suppression. *Trichoderma* treatment were known to produce chitinase and β (1-3, glucocinase) enzymes which could degrade the cell wall leading to the lysis of hyphae of the pathogen as noted by Wu *et al*. (1986). *Trichoderma* sp. attacks to the host hyphae via coiling hooks and appressorium like bodies and penetrate the host cell wall by secreting lytic enzymes (Kubicek *et al*., 2001). β-1, 3-Glucanases and chitinases have been found to be directly involved in the mycoparasitism interaction between *Trichoderma* sp. and its hosts (Kubicek *et al*., 2001). Benitez *et al*. (2004) reported that the *Trichoderma* attacks to the pathogen via cell wall carbohydrates. Once it is attached, it coils around the pathogen and forms the appresoria. The next step consists of the production of all cell wall-degrading enzymes and peptaibols. The *Trichoderma* treatment effect was found significant ($p = 0.05$) in respect of wilt incidence and plant growth during the experimentation (Dubey *et al*., 2007; Federico *et al*., 2007; Mousseaux *et al*., 1998).

CONCLUSION

Reduction of *F. oxysporum* infection in *C. infundibuliformis* var. Danica was achieved by the use of *Trichoderma* isolates. *Trichoderma* treatment also increased growth of plants. The results of this study clearly revealed the potential of *Trichoderma* as a biological agent to control *F. oxysporum* wilt on *C. infundibuliformis.*

ACKNOWLEDGMENTS

The authors are grateful to Mr. Arne Svinningen, Chairman, Managing Director, Green Farms Ltd., Marawila, Sri Lanka for his technical help, writing assistance and provided and cared for study through-out the research period. Equally the authors wish to thank Mr. Arne Svinningen, Chairman, Managing Director, Green Farms Ltd., Marawila, Sri Lanka for his Financial and material support in conducting this study. The research was fully supported (Financial and material support) by Green Farms, Ltd., Marawila. The authors also appreciate Mr. M.D.S.D. Karunaratne, Technical Manager, Ms. K.P. Rashani and Staff members, Laboratory unit, Green Farms Ltd, for their support to collected data through-out the research period.

REFERENCES

Almeida, F.B.R., F.M. Cerqueira, R.N. Silva, C.J. Ulhoa and A.L. Lima, 2007. Mycoparasitism studies of *Trichoderma harzianum* strains against *Rhizoctonia solani*: Evaluation of coiling and hydrolytic enzyme production. Biotechnol. Lett. 29: 1189-1193.

Anonymous, 2006. *Trichoderma*: Systematic mycology and microbiology. ARS, USDA and Department of Plant Pathology, Penn. State University. http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm

Bashar, M.A. and B. Rai, 1994. Antagonistic potential of root region micro flora of chickpea against *Fusarium oxysporum* f. sp. *ciceri*. Bangaladesh J. Bot., 23: 13-19.

Benitez, T., A.M. Rincon, M.C. Limon and A.C. Codon, 2004. Biocontrol mechanisms of *Trichoderma* strains. Int. Microbiol., 7: 249-260.

Bisset, J., 1991. A revision of genus *Trichoderma* II: Infrageneric classification. Can. J. Bot., 69: 2357-2372.

Brunner, K., S. Zeilinger, R. Ciliento, L.S. Woo, M. Lorito, C.P. Kubicek and L.M. Robert, 2005. Improvement of the fungal biocontrol agent *Trichoderma atroviride* to enhance both antagonism and induction of plant systemic disease resistance. Applied Environ. Microbiol., 71: 3959-3965.

Datta, B.S., A.K. Das and S.N. Ghosh, 2004. Fungal antagonists of some plant pathogens. Mycopathology, 1: 15-17.

Dubey, S.C., M. Suresh and B. Singh, 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp.*ciceris* for integrated management of Chickpea wilt. Biol. Control J., 40: 118-127.

Engelhard, A.W., 1997. Evaluation of fungicide for control of ascochyta blight of field grown *Chrysanthemums*. Methods Evaluating Pesticides Control Plant Pathogens, 2: 237-239.

Federico, G.R., M.M. Reynoso , M. Ferez, S.N. Chulze and A.M. Torres. 2007. Biological control by Trichoderma species of Fusarium solani causing peanut brown root rot under field conditions. Crop Prot., 26: 549-555.

García, P.G., A.M. Pascuas and E.G. de Granada, 1997. Effect of two *Trichoderma* spp. isolates on *Fusarium oxysporum* f. sp. *Dianthi* in carnation (*Dianthus caryophyllus*). International Symposium on Cut Flowers in the Tropics. ISHS Acta Horticulturae 482. http://www.actahort.org/books/482/482_22.htm.

Gilardi, G., M. Baudino, M.L. Gullino and A. Garribaldi, 2008. Attempts to control fusarium root rot of bean by seed dressing. Commu. Agric. Applied Biol. Sci., 73: 75-80.

Hong, J.K. and B.K. Hwang, 1998. Influence of inoculums density, wetness duration, plant age, inoculums method and cultivar resistance on infection of pepper plants by *Colletotrichum coccides*. Plant Dis., 82: 1079-1083.

Howell, C.R., 2003. Mechanism employed by *Trichoderma* species in the biological control of plant diseases. The history and evolution of current concepts. Plant Dis., 87: 4-10.

Ilan, C., Y. Brotman and A. Viterbo, 2009.. *Trichoderma*-An environmentally friendly biocontrol agent of plant disease. Proceedings of the 5th International Conference on Biopesticides: Stakeholders' Perspectives, Apr. 26-30, New Delhi, pp: 1-1.

Irfan, Y.S. and A.N. Khalid, 2007. *In vitro* biological control of *Fusarium oxysporum*-r causing wilt in *Capsicum annum*. Mycopathology, 5: 85-88.

Kubicek, C.P., R.L. Mach, C.K. Peterbauer and M. Lorito, 2001. *Trichoderma*: From genes to biocontrol. Plant Pathol., 83: 11-24.

Larry, A.N., 1997. Use of statistics in planning data analysis and interpretation of fungicides and nematicides test. Methods Evaluating Pesticides Control Plant Pathogens, 2: 11-23.

Lieckfeldt, E., G.J. Samuels, H.I. Nirenberg and O. Petrini, 1999. A morphological and molecular perspective of *Trichoderma viride*: Is it one or two species. Applied Environ. Microbiol., 65: 2418-2428.

Mousseaux, M.R., R.K. Dumroese, R.L. James, D.L. Wenny and G.R. Knudsen, 1998. Efficacy of *Trichoderma harzianum* as a biological control of *Fusarium oxysporum* in container-grown Douglas-fir seedlings. New Forests, 15: 11-21.

Mujeebur, K., R. Shahana, M. Khan and F.A. Mohiddin, 2004. Biological control of Fusarium wilt of chickpea through seed treatment with the commercial formulation of Trichoderma harzianum and/or Pseudomonas fluorescens. Phytopathol. Mediterr., 43: 20-25.

Mukhopadhyay, A.N., 2009. Challenges, changes and choices before Trichoderma based biopesticides. Proceedings of the 5th International Conference on Biopesticides: Stakeholders' Perspectives. New Delhi.

Ozbay, N. and S.E. Newman, 2004. Biological control with *Trichoderma* spp. with emphasis on *T. harzianum*. Pak. J. Biol. Sci., 7: 478-484.

Reid, T.C., M.K. Hausbeck and K. Kizilkaya, 2002. Use of fungicides and biological control in the suppression of *Fusarium* crown and root rot of asparagus under green house and growth chamber conditions. Plant Dis., 86: 493-498.

Riley, M.B., M.R. Williamson and O. Maloy, 2002. Plant disease diagnosis. The Plant Health Instructor. 10.1094/PHI-I-2002-1021-01

Rubio-Perez, E., M.L. Molinero-Ruiz, J.M. Melero-Vara and M.J. Basallota-Urebra, 2008. Selection of potential antagonistic against asparagus crown and root rot caused by *Fusarium* sp. Commu. Agric. Applied Biol. Sci., 73: 203-206.

Samuels, G.J., O. Lieckfeldt and C.P. Kubick, 1998. The *Hypocrea schweinizii* complex and *Trichoderma* sect. *Longibrachiatumi*. Stud. Mycol., 41: 1-54.

Shanmugam, V., V. Sharma and Ananthapadmanaban 2008. Genetic relatedness of *Trichoderma* isolates antagonistic against *Fusarium oxysporum* f. sp. *dianthi* inflicting carnation wilt. J. Folia Microbiol., 53: 130-138.

Singh, R., B.K. Singh, R.S. Upadhyay, B. Rai and Y.S. Lee, 2002. Biological control of *Fusarium* wilt disease of pigeonpea. Plant Pathol. J., 18: 279-283.

Singh, A., N. Sharma and H.B. Singh, 2004. Effect of UV light on the bio control potential of *Trichoderma harzianum*. Indian Plant Pathol., 22: 56-58.

Watanabe, T., 2002a. *Fusarium* sp. Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species. 2nd Edn., CRC Press, New York.

Watanabe, T., 2002b. *Trichoderma harzianum*: Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species. 2nd Edn., CRC Press, New York.

Wu, W.S., S.D. Liu, Y.C. Chang and J. Tschec, 1986. Hyperparasitic relationships between antagonists. Plant Prot. Bull., 28: 91-100.