POTENTIAL BIOCIDE OPTIONS AND BIOLOGICAL CONTROL AGENT FOR *Ceratocystis paradoxa* ISOLATED FROM COCONUT GROWING AREAS OF SRI LANKA

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ABSTRACT

Ceratocystis paradoxa is a pathogen causing stem bleeding, leaf and fruit rot in variety of crop plants including coconut. Since C. paradoxa is associated with coconut trees, there is an opportunity for the pathogen to be existed in coir and coir products as well. According to the quarantine requirements, this pathogen has to be free in coir and coir products that are exported from Sri Lanka. During this study the pathogen was isolated from the samples of coir collected from island wide coconut growing regions of Sri Lanka. Colony morphology and spore morphology were compared among these isolates. Bio-control and the sensitivity of the isolates to the antagonist were evaluated against Trichoderma viride. The biocide effects were evaluated against methyl bromide fumigation, formaldehyde fumigation and water vapor heat treatment. According to the results the pathogen was present in all the samples collected from different locations of the coconut growing regions in Sri Lanka. The isolated colonies grown on Potato Dextrose Agar (PDA) plates produced two asexual spores; the endoconidia and chlamydospores. Endoconidia are formed in the conidiophores making long chains. The spore morphology complies with available literature that characterized C. paradoxa. Biological control of the pathogen using an antagonist; T. viride is equally effective for all the isolates as the biological control agent by killing the vegetative growth and losing the spore viability. Formaldehyde fumigation is proved as an alternative to the methyl bromide fumigation for elimination of C. paradoxa in coir products. The water vapor heat treatment when full-core temperature of coir dust matrix is reached up to 70 ^{o}C the viable spores of the pathogen are destroyed.

Key words: *Ceratocystis paradoxa, Thielaviopsis paradoxa,* Coconut stem bleeding, *Trichoderma viride*. biological control agents

Introduction

Ceratocystis paradoxa is a plant pathogen, that infects palm trees including *Cocos nucifera* (coconut) and many other plantation crops such as pineapple, sugarcane etc. An asexual reproductive stage (anamorph) of the pathogen is known as *Thielaviopsis paradoxa*. Due to the pathogen infection in coconut, the plant trunk either collapses on itself or the canopy suddenly falls off without warning. A common symptom observed is "stem bleeding; a reddish-brown stain that runs from the point of infection making a wound. The tissue surrounding the wound is quite soft in comparison to the surrounding trunk tissue. Eventually, the trunk is collapsed at the point of infection (Paulin-Mahady et al., 2002). For the first time the disease was reported in Sri Lanka by Petch in 1906 (Warwick and Passos, 2009).

Sri Lanka exports coir and coir products that have a constant share of the country economy. Therefore, it is important to ensure the products quality up to the standards required for the export market. Accordingly, the pathogen should be free from the product prepared for the export market. However, prevalence of *C. paradoxa* in Sri Lanka at the level of high incidence can cause heavy contaminations in coir and coir products produced for importation (Jayaratne et al., 2015. For the elimination of the pathogen, methyl bromide fumigation is the most common treatment used by coir product exporters and plant quarantine authorities. At present, methyl bromide is considered as an environmental hazard especially ozone depleting chemical (Dharmawardana, 2014), the alternative treatments are essentially needed. Formalin is widely used chemical alternative for methyl bromide in order to eliminate fungal and bacterial contaminations in various industrial applications. It is normally diluted with water 1:50–100, at a rate of 10 liters/m^2 for the control of bacteria and fungi in soil (Lambrada, 2007). However, coir product exporters and quarantine authorities are reluctant to use formaldehyde fumigation that may be due to the lack of confidence over the treatment outcomes. Apart from that, low humidity heat at 85 ^{O}C is one of the physical treatments which maintain a rate of core temperature for 15 continuous hours with 40% relative humidity. Autoclaving is also used which maintains the core temperature at 121^{O}C for 30 minutes at 100 kilopascals (Ritchie 2009). Since, these heat treatments are costly and laborious, the validation and introduction of simple and most economical heat treatment options should be needed.

Biological control is an eco-friendly, long-lasting, highly effective method for the elimination of soil borne pathogens like *C. paradoxa* and a good alternative for the chemical and physical treatments. The literature revealed that the pathogen can be

successfully controlled by *Trichoderma viride* (Jayaratne et al., 2015; Tapwal1 et al., 2011). The biological control ability of *T. viridae* is due to several factors. *T. viridae* produces several groups of antibiotics, toxins and then the growth of the pathogen is inhibited (Eziashi et al., 2010). Apart from that the direct attack is called mycoparasitism which kills the pathogen by mechanical and chemical means. Also *Trichoderma* species can inhibit or reduce the growth of the pathogen through competition for space, nutrients or oxygen. *Trichorderma* is fast growing and has the ability to colonize on a wide variety of substrates. This makes *Trichorderma* efficient soil colonizers and bio-control agents. (Sanchez et al., 2007).

Thus the aim of this study was to evaluate the bio control capability of *Trichoderma viridae* and other alternative chemical and physical treatment methods instead of methyl bromide fumigation for the elimination of *C. paradoxa* in coir and coir products produced for the export market. Accordingly, the study was carried out on the following specific objectives such as proper identification of the pathogen and its biological control agent and the effectiveness of the biological control agent to eliminate the pathogen, validation of formaldehyde treatment and water vapor heat treatment in contrast to the existing chemical and physical treatments used in quarantine applications.

Methodology

Sample collection: Coir and coir dust samples were collected from sixteen locations in North Western province and Western province of Sri Lanka where the prevalence of extensive coconut cultivation and coir based industries located in the Island. The positive control reference culture of *C. paradoxa* was obtained from the microbial culture collection of the National Plant Quarantine Service (NPQS), Colombo.

Isolation and cultivation of the pathogen: The dilution plate technique was used to isolate the pathogen from coir and coir dust samples into Potato Dextrose Agar (PDA) plates. The well separated colonies were sub-cultured on new PDA plates for identification and enumeration of the isolates.

Morphological variations of the different isolates: The slide culture technique and sticky tape technique were used to determine the undisturbed mycelial and spore morphology of the isolates. The ocular micrometer method was used to measure the size of endoconidia, chlamydospores, conidiophores and vegetative mycelia of the isolates. The isolates were identified as using the key outlined by Coomaraswamy (1979).

Molecular characterization of the isolates: DNA from the isolates was extracted according to the method described by Jayaratne et al., 2012. To establish the taxonomic status of the isolates the PCR amplification of internal transcriber spacers of the rDNA gene (ITS1 and ITS2) was carried out for the isolates followed by restriction fragment length polymorphism (RFLP) analysis. These are rapid and reliable techniques for the accurate identification of fungi.
Amplification of the ITS regions and the 5.8 S ribosomal gene was obtained using the ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTATTGATATGC3') universal primers (White et al., 1990). Amplification was performed in thermal cycler (Techne TC-412) at an initial duration 95 ^{O}C for 1 min and programmed for 30 cycles of denaturation at 95 ^{O}C for 1 min, annealing at 55 ^{O}C for 1 min and elongation at 72 ^{O}C for 2 min followed by final extension at 72 ^{O}C for 10 min. PCR was performed in 25 µl reaction mix containing 1 x buffer supplied with the enzyme, 2mM $MgCl_2$, 1µM of each primer (ITS1 and ITS2) supplied by Integrated DNA Technologies, 100 µM dNTPs (Promega), 0.5 units of *Taq* DNA polymerase (Promega) and 25 ng of template DNA.
Restriction profiles of the amplified DNA were generated using *Alu*I endonuclease (Promega). Agarose gel electrophoresis was performed to visualized ethidium bromide gel under UV transilluminator and gel images were obtain for phylogenetic analysis that was carried out using GelCompar II software and confirmed the identity of the isolates as *C. paradoxa*.

Isolation and identification of biological control agent: *Trichoderma*: The *Trichoderma* spp were isolated from coir dust and soil samples collected from Coir Research and Development Institute (CRDI), Lunuwila of North Western Province of Sri Lanka. The method of isolation and cultivation were same as the methods described for the "Isolation and cultivation of the pathogen". The isolates were characterized by using the identification key published by Coomaraswamy et al., (1981) and confirmed the identity using the *Trichoderma* interactive key provided in the website http.//nt.arsgrin.gov/taxadescriptions/key/framekey.cfm ?gen=Trochoderma. Accordingly, morphological as well as genomic features of the isolates have been considered for the correct identification of the biological control agent as *T. viridae*.

Mycoparasitism of *C. paradoxa* isolates by *T. viridae* was studied using the dual culture technique developed by Dennis and Webster (1971) described by Sanchez et al., 2007. The technique allows the researchers to understand the overall effect of biological control agents. The inoculated PDA plates containing dual cultures were incubated at room temperature (28 ± 1℃) under condition of light for 7 days. The time for the first contact between the antagonist and the pathogen, and the advance of antagonism over the pathogen colony were measured. Control plates were prepared by inoculating *C. paradoxa* without the antagonist; *T. viridae*.

Antagonistic effect on spore viability: The spores of *T. viridae* and *C. paradoxa* isolates were scraped off from the PDA plates using sterile glass slides separately. The spore suspensions were prepared in sterile distilled water making the spore concentrations 10^7CFU/ml in each suspension. The spore suspensions of *C. paradoxa* isolates were individually mixed with equal volumes of the spore suspension of *T. viridae*. A volume of 0.1ml from each of the mixed spore suspensions were poured onto PDA plates separately and spread evenly on the agar **s**urface using sterile glass spreaders. The plates were incubated at room temperature (28 ± 1℃) for two days. The spore germination and mycelial growth of *T. viridae* and *C. paradoxa* isolates were observed on PDA plates in hourly intervals.

Effect of diffusible non-volatile metabolites produced by the antagonist on the mycelial growth of the pathogen isolates: The cellophane membrane method proposed by Dennis and Webster (1971) described in Sanchez et al., 2007 was used to determine the effect of biocides produced by the antagonist to control the growth of *C. paradoxa*. A sterile cellophane membrane (9 cm diameter) was placed on the surface of PDA plates containing 25 ml of the medium. A disc of 5 mm mycelium from actively growing edges of a 3-day old colony of *T. viridae* was placed in the center of each PDA plate. The Petri plates were then incubated for 2 days at 37°C in dark. After the incubation, the cellophane membranes with the fungal mycelia were removed from each plate. The 5 mm mycelial discs, taken from actively growing edges of 3-day old colonies of *C. paradoxa* isolates were separately placed on the center of each petri plate in the same position where the antagonist; *T. viride* isolate was located. The plates were again incubated at 37°C in dark for 5 days. The diameter of each fungal colony was measured. The controls were prepared by placing 5 mm mycelia disc of *C. paradoxa* instead of the antagonist. Each treatment was replicated four times.

FUMIGATION OF COIR DUST INOCULATED WITH SPORE SUSPENSIONS OF THE PATHOGEN: This study was carried out in small chambers consisted of similar conditions to the large fumigation treatment units used in NPQS of Sri Lanka. Prior to the fumigation, sterilized coir dust 5 g samples in Petri plates were inoculated by 1 ml spore suspension containing $4x10^4$ spore/ml and mixed well. Then the inoculated coir samples in the Petri plates were placed in three separate chambers of the fumigator labeled as Methyl bromide, Formaldehyde and control. The fumigation treatment for 24 hours was then performed in each chamber. After the degassing procedure coir samples were withdrawn from the chambers. The dilution plate technique was used to determine the CFU of *C. paradoxa* produced by remaining viable spores after each treatment.

Water vapor heat treatment for elimination of the pathogen in coir dust: Sterilized coir dust 50 g samples were inoculated with 10 ml of spore suspension containing 4 x 10^4 spores/ml. The coir samples were mixed well and then placed in a sterile metal steamer. A thermometer was placed in contacting with the core of the coir dust to detect the temperature. The heat was applied by using a hot plate.

Different time-temperature combinations were given to inoculated coir dust. Accordingly, 55℃, 70℃ and 85℃ temperatures in combination with 5 min, 10 min, 20 min and 30 min time durations were used for the treatments. When the temperature reached respective time/temperature combinations, 1 g of coir dust was taken from the apparatus and enumerated the *C. paradoxa* population in each sample according to the dilution plate count technique.

Results and Discussion

According to the quarantine requirements coir and associated products should be free from *C. paradoxa*. But the present investigation revealed that the samples collected from the coconut growing regions in Sri Lanka are contaminated with the pathogen identified as *C. paradoxa*. The population density of the pathogen varied from 10 to 100 CFU/g or more in different samples (Table 1).

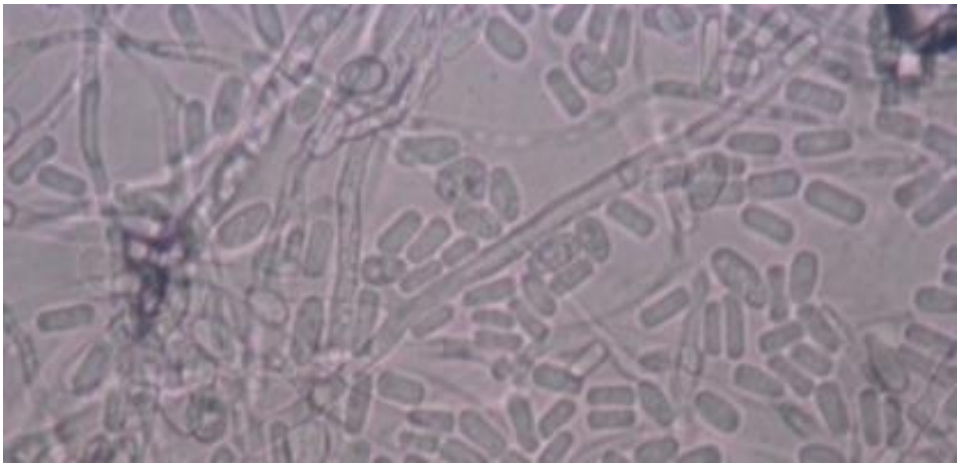
Table 1: The average population densities of the pathogen in the coconut growing regions in Sri Lanka

Province	Location	CFU/g of a sample
North-Western	Lunuwila	40 to > 100
	Dunkannawa	60 to > 100
	Kurunegala	10 to > 100
Western	Mirigama	30 to 50

The infection of the pathogen was reported in 1906 by Petch in the coconut growing regions of Sri Lanka stating that the stem bleeding disease of coconut caused by *C. paradoxa* was first reported in the country (Dulce et al., 2009). Moreover, the prevalence of the pathogen in the sugarcane growing areas of the country was also recorded by Egan in 1961. These historical evidences are sufficient to believe that the island wide distribution of the pathogen with or without causing the disease in palm trees and other susceptible hosts. These results revealed that the population density of *C. paradoxa* is highest in North-Western province where the coconut growing triangle is laying in the island. The parallel evolution of the pathogen with its natural hosts may not cause severe infections in these locations but survives causing mild infections and sporadic disease incidences. However, this situation makes high inoculum potential of the pathogen in the crop environment leading to serious problems in coir and coir product exportation from Sri Lanka.

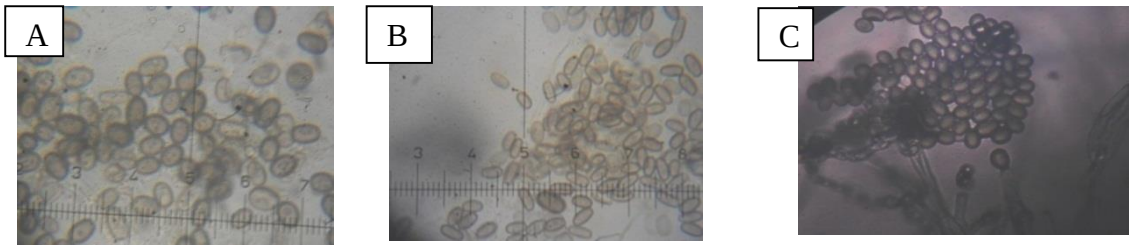
Morphological variations of the pathogen isolates: The pathogen isolated into PDA produced two different types of asexual spores; endoconidia and chlamydospores. There were no differences in the structure of endoconidiophores of all the isolates. The endoconidiophores got enlarged at the base when releasing endoconidia. All the isolates produced hyaline, cylindrical, thin walled endoconidia; 9-12 µm in length and 4.8-7.2 µm in width (Figure 1). These sizes comply with the size range stated in the published literature (Moutia & Saumtally, 1999 and Ploetz, 2008)

Figure 1: The endoconidia and the vegetative mycelium of *C. paradoxa*



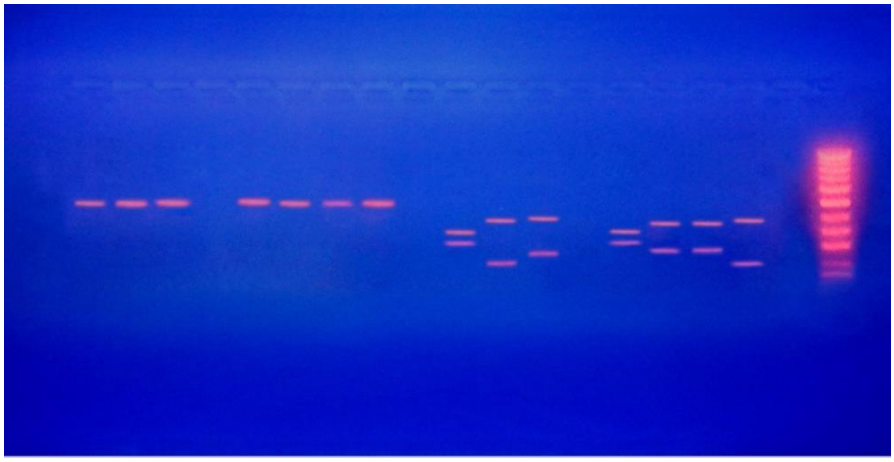
The chlamydospores produced by the isolates grown in PDA are thick walled, light brown to dark brown and basically oval in shape. They were produced in older cultures ensuring the long-term survival of the pathogen in soil (Ploetz, 2008; Moutia & Saumtally, 1999). The oval shape is prominent in Kurunegala isolates and Mirigama isolates. However, Lunuwila and Dunkannawa isolates produced oblong chlamydospores that are distinct from the above mentioned isolates. The reference culture Rf 17 collected from National Plant Quarantine Service Colombo, produced slightly oval chlamydospores (Figure 2, A, B and C). Accordingly, three different morphological types of chlamydospore are produced by these isolates. All the isolates produce chlamydospores in the range of 9.6-16.8 µm length and the range of 7.2-9.6 µm width, that comply with the characteristic features of *Ceratocystis paradoxa* (Moutia & Saumtally, 1999 and Coomaraswamy, 1979). These observations are used for authentic identification of the pathogen.

Figure 2: The chlamidospore morphology of the *Ceratocystis paradoxa* isolates, (A) Oval chlamydospores; (B) Oblong chlamydospores; (C) Slightly oval chlamydospores



Molecular characterization of *Ceratocystis paradoxa* isolates: Amplification product of internal transcriber spacers of the rDNA gene (ITS1 and ITS2) were produced for seven isolates obtained from North Western Province; Lunuwila, Dankotuwa and Kurunagala. The PCR amplified DNA size for these isolates were approximately 590 bp that comply with the size of the PCR amplified product of ITS region of *C. paradoxa* genome as stated in the literature (Alvarez *et al*., 2012).

Figure 3: The gel image of PCR amplified DNA for ITS region of *C.paradoxa* genome and RFLP patterns of different isolates generated by *Alu 1* digestion of the PCR amplified DNA

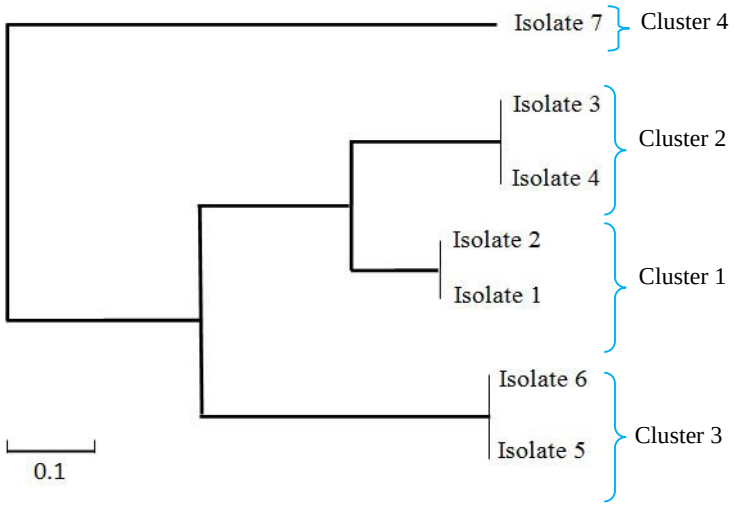


PCR amplified DNA of ITS region: Lane 1, isolate 1; Lane 2, isolate 5; Lane 3, isolate 7; Lane 4, Negative Control; Lane 5, isolate 2; Lane 6, isolate 3; Lane 7, isolate 4; Lane 8, isolate 6
RFLP pattern of *Alu*I digested PCR amplified DNA of ITS region: Lane 10, isolate 1; Lane 11, isolate 5; Lane 12, isolate 7; Lane 13, Negative Control; Lane 14, isolate 2; Lane 15, isolate 3; Lane 16, isolate 4; Lane 17, isolate 6; Lane 19, 100 bp marker DNA

Restriction digestion with *Alu*I revealed four different restriction patterns. Isolates 1 and 2 have similar restriction pattern while isolates 5 and 6, isolates 3 and 4 also have the similar restriction patterns. And isolate 7 has a restriction pattern which differs from others as well. So this confirms that all these seven isolates contain four different genotypes of the *Ceratocystis paradoxa.* This is further supported by the literature (Martin *et al*., 2005).

Phylogenetic analysis of these fungal isolates was carried out using GelCompar II software; based on the RFLP patterns of different isolates appeared on the gel image (Fig 3). After the gel image processing and the cluster analysis; a dendrogram was obtained (Fig 4). According to the dendrogram constructed for different isolates of *Ceratocystis paradoxa*, the isolates 1 and 2 seem to be more phylogenetically related, while isolates 3 and 4 also have a close phylogenetic relationship. Likewise Isolates 5 and 6 also have a close phylogenetic relationship. Isolate number 7 is the most divergent one, among the other studied isolates. Accordingly, these information are used for the confirmation of the identity of the pathogen and discrimination of its genotypes.

Figure 4: The dendrogram constructed for the different isolates of the *C. paradoxa* using similarity matrix and "UPGMA" clustering algorithm.



Mycoparasitism of *T. viride* on *C. paradoxa*: The time taken for the first contact of *T. viride* with all different *C. paradoxa* isolates was 48 hours indicating that the radial growth rate of *T. viride* is equal in all combinations in the dual cultures. It was also observed that the growth of *C. paradoxa* isolates lessened as soon as they first contacted with *T. viride* which then rapidly overgrew on these colonies. The complete invasion and sporulation of *T. viridae* occurred after 6-7 days of incubation at room temperature (28 ± 1℃). Mycoparasitism towards pathogenic fungi by *Trichoderma* species is proposed as a mechanism of bio control. For this ability several enzymes are involved. The prominent mechanism is that the degradation of cell wall constituents, prior to lysis the cells before chemotropic growth and coiling take place (Eziashi., et al., 2010; Chet et al., 1998).

Table 2: Dual cultures antagonistic effect of *T. viride* vs *C. paradoxa* isolates

Original location	Isolate Number	Percentage Inhibition		
		Day 5	Day 6	Day 7
North Western province Lunuwila/Dunkannawa	1	*86*	93	100
	2	86	93	100
	3	78	93	100
	4	86	93	100
	5	86	93	100
North Western province Kurunagala	6	78	100	100
	7	93	100	100
	8	86	100	100
	9	78	100	100
	10	71	93	100
	11	86	100	100
Western province Mirigama	12	86	100	100
	13	86	100	100
	14	86	100	100
	15	86	100	100
	16	86	100	100

Each data point represents the mean of four replicates. The P-value of location 0.052 is greater than 0.05 but less than 0.10. Therefore, the location effect is significance at 10% level

Although the first contact between the pathogen and the antagonist seems similar, the final completion of inhibition is not at all the same. The samples obtained at same locations showed similar time for the complete inhibition (Table 2). Accordingly, the isolates from Lunuwila and Dunkannawa did not show complete inhibition up to seven days while the isolates from Kurunegala and Mirigama took six days only for the complete inhibition except one isolate from Kurunagala. The interaction of antagonist; *T. viride* and *C. paradoxa* shows correlation with their original location. It seems that the antagonist and *C. paradoxa* isolated from same geographical location has low virulence interaction than to the *C. paradoxa* isolated from different geographical locations. This phenomenon can also be explained by coevolution of antagonist and pathogen living in the same geographical location similar to that the coevolution of host and pathogen. These observations suggest that the alien biological control agents may be more effective than the indigenous ones for biological control of plant pathogens.

Antagonistic effect of *T. viridae* on spore viability of *C. paradoxa*: In the controls the germination of chlamydospores started 7 hours after the inoculation. A considerable growth of hyphae was seen after 12 hours. In the mixed spore suspension both *C. paradoxa* spores and *T. viridae* spores were visible, but no germination was found. The subsequent results showed that only the *T. viridae* spores germinated when both were in the same suspension. The photographs taken in hourly intervals showed that only *Trichoderma* hyphae lengthened and chlamydospore of *C. paradoxa* did not show germination and growth.
According to the available literature, genus *Trichorderma* produces a variety of volatile and nonvolatile fungicidal metabolites making inhibitory effect on the germination of *Ceretocystis* spores (Tapwal1 et al., 2011; Dix and Webster, 1995). It has also been reported that *Trichoderma* can compete well with *C. paradoxa* for space, substrates, nutrients, and/or oxygen and the antagonist can win the competition easily inhibiting the others due to their fast growing nature (Bourguignton, 2008). This is one of the other reasons that *Ceretocystis* spores did not germinate, as the necessary conditions for their spore germination was inhibited by the competitive *Trichorderma viride*.

Effect of nonvolatile metabolites produced by *T. viride* on *C. paradoxa*: All isolates showed equal inhibition during 5 days of incubation in dark showing no mycelial growth of *C. paradoxa* isolates unlike in the control plates in which *C. paradoxa* isolates showed confluent growth on the entire PDA plate at 5 days of incubation at room temperature (28 ± 1℃). *T. viride* completely inhibited the mycelial growth of *C. paradoxa* isolates at 12 days of exposure. Therefore, it was assumed that a diffusible non-volatile substance was responsible for the inhibition of *C. paradoxa* growth. The observation of this study is agreeable with Bourguington, 2008 who stated that species of *Trichoderma* produces nonvolatile metabolites, such as antibiotics and enzymes, which involve in inhibiting growth of pathogenic fungi and spore germination.

Effect of formaldehyde fumigation and methyl bromide fumigation on *C. paradoxa* in coir dust: According to NPQS Sri Lanka guidelines, the mixing ratio of 37% formalin and potassium permanganate is 2:1 for formaldehyde fumigation i.e., 42 ml formalin mixed with 21 g potassium permanganate in the formalin fumigation chamber. The dose of methyl bromide is 48 g/m^3 in the methyl bromide fumigation chamber. The dilution plate count experiment showed that no growth after 24 hours of formaldehyde fumigation and methyl bromide fumigation whereas control experiment showed 3.09 x 10^3 CFU of *C. paradoxa* per gram of coir dust. Accordingly, both treatments are equally effective for eradication of *C. paradoxa* in coir dust. Moreover, these observations confirmed that the formaldehyde fumigation is a reliable and effective alternative to the currently practicing methyl bromide fumigation for eradication of *C. paradoxa*.

Effectiveness of water vapor heat treatment for the elimination of *C. paradoxa* in coir dust: Ritchie (2009) has described a water vapor heat treatment method currently used for elimination of *C. paradoxa* from coir products. Accordingly, the core temperature at 85℃ for 15 continuous hours with 40% RH can effectively eliminate *C. paradoxa* in coir products. However,

Berg (1926) showed that hot water treatment at 51℃ for 30 minutes eliminate *C. paradoxa* in infected sugarcane stem cuttings used for planting.

The present study revealed that the water vapor heat treatment given to the coir dust inoculated with *C. paradoxa* spores showed the reduction of spore viability when the temperature reached at 55℃. The temperature at 55℃ for 20 minutes exposure can eliminate almost all the viable spores in the inoculated coir dust. Whereas 70℃ and above showed no escapees of viable spores (Table 3). Moreover, this is a cost-effective and speedy treatment in contrast to the water vapor heat treatment currently used by quarantine authorities and coir exporters.

Table 3: The effect of water vapor heat treatment on viability of *C. paradoxa* spores in inoculated coir dust.

Temperature	Growth of *C. paradoxa* CFU/g of coir dust after the heat treatment exposure at different temp/time durations			
	5 min	10 min	20 min	30 min
55℃	2.68×10^3	2.00×10^3	nil	nil
70℃	nil	nil	nil	nil
85℃	nil	nil	nil	nil
100℃	nil	nil	nil	nil
Control	3.09×10^3			

Conclusion

The experimental results revealed powerful antagonistic effects of *T. viride* on all the isolates of *C. paradoxa*. The complete inhibition of chlamydospore germination by the germinating conidia of *T. viride* in the mixed spore suspension, the overall invasion of *C. paradoxa* isolates by *T. viride* after 6-7 days of incubation at room temperature in the dual cultures and killing the vegetative growth of the isolates by the nonvolatile or diffusible metabolites released by *T. viride* suggest that there is a strong possibility to use *T. viride* as the antagonist to eliminate the pathogen from the raw materials used to produce coir and coir product for the export market. The formaldehyde fumigation is equally effective as methyl bromide fumigation for the elimination of *C. paradoxa* in coir dust. As an alternative low-cost method water vapor heat treatment at 70℃ core temperature for 5 min destroys the viable spores of *C. paradoxa* in coir dust.

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