Biological control of *Thielaviopsis* Bud Rot of *Hyophorbe lagenicaulis* in the field

Kasem Soytong[1*], Wattanachai Pongnak[1] and Hattachai Kasiolarn[1]

[1]Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok 10520,Thailand

Soytong, K., Pongak, W. and Kasiolarn, H. (2005). Biological control of *Thielaviopsis* bud rot of *Hyophorbe lagenicaulis* in the field. Journal of Agricultural Technology 1 (2): 235-245.

Thielaviopsis Bud Rot was first reported to infect the Bottle Palm (*Hyophorbe lagenicaulis*) in Thailand and causes a range of symptoms e.g. Stem Bleeding, Bitten Leaf, Dry Basal Rot and Heart Rot. The isolated pathogen is confirmed to be pathogenic to the Bottle Palm. We carried out bi-culture antagonistic assays using the antagonistic fungi, *Chaetomium cupreum* and *C. globosum* against *T. paradoxa*. Assays showed these fungi to be antagonistic against the pathogen *in vitro*. In the field the five year old Bottle Palms completely recovered from disease when *Chaetomium* biological product was applied to infested soil at the rate of 20 g/plant. Antagonistic substances produced by *C. cupreum* and *C. globosum* were sprayed to control terminal bud rot and integrated with other cultural control measures. The treated trees recovered significantly within 30 days of application and new leaves emerged. The *Chaetomium* biological product has good potential in the control of Bud Rot of Bottle Palm.

Key words: *Chaetomium cupreum, C. globosum,* disease, ornamentals, palms

Introduction

The Bottle palm (*Hyophorbe lagenicaulis*) is an intriguing palm from the Mascarene Islands which obtains its name from the unusual bloated trunk which in some specimens resembles a bottle. Also distinctive are the dark green pinnate fronds, which have a characteristic, prominent twist, and the crown, which consists of a small number of, expanded fronds (usually four to six). The bottle palm is rather cold sensitive and is optimally suited to tropical regions, although they can succeed in a warm position on the subtropics (Jones, 1995). *Hyophorbe lagenicaulis* is much prized in cultivation for its curious bottle-shaped trunk. It has a single stem which reaches 6 m in height and up to 70 cm in diameter. The inflorescences are 75 cm long with a spread of up to 50 cm. It is easily cultivated in the tropics and warm subtropics, but is sensitive to

[*]Corresponding author: Kasem Soytong; e-mail: kskasem@kmitl.ac.th

even very brief periods of temperature below 4ºC. Germination can take 2-3 months and seedlings should be ready for planting within 2 years. *Hyophorbe lagenicaulis* is a slow-growing species that when well established grows at a rate of 25-30 cm of trunk/year until it starts to flower (Dransfield, 1996).

Thielaviopsis bud rot is caused by *Thielaviopsis paradoxa* (teleomorph = *Ceratocystis paradoxa*) which is a serious disease of various species of palms e.g. *Areca catechu*, *Elaeis guineensis*, *Hyophorbe lagenicaulis Phoenix africanus*, *Rhapis* sp., *Roystonea elata*, *Sabal palmetto*, *Syagus romanzoffiana* and *Washingtonia filifera* (Chase and Broschat, 1993). The disease has increased in importance with the expansion of palm cultivation and in Libya may cause 50% losses in young plantations (Gariani *et al*., 1994). Chase and Broschat (1993) reported that avoidance of wounds of palms grown in the field or nurseries can limit disease incidence. Infected palms should be cut, removed, and destroyed. Localized infections can be excised through tree surgery followed by benomyl treatment. Although some resistance to this pathogen has been documented in the Date palm, the range of disease reaction is not know for ornamental palm species. Early infection of bud, leaf or root tissue can be effectively treated by benomyl. Mubarak *et al*. (1994) reported that an isolate of *Thielaviopsis paradoxa* (as *Ceratocystis paradoxa*) from diseased Date palms grew and sporulated on PDA or malt extract agar with an optimum temperature of 30ºC.

Application of chemical fungicides has been recognized to cause environmental pollution and leave chemical residues in the soil, water and agricultural products, and it is known that the continuous use of chemical fungicides leads to the development of resistance in some pathogens (Soytong and Soytong, 1996). Biological control of plant pathogens is a recent successful strategy for disease control and has successfully been integrated with other control measures. Biological control methods can reduce the heavy use of chemical fungicides, improving the agro-ecosystem and maintaining a natural balance (Soytong *et al*., 1999b). There are several reports on the potential use of biological control agents against plant pathogens. *Chaetomium* species are strictly saprobic antagonists and have been shown to be against several plant pathogens, e.g. *Botrytis cinerea* (Kohl *et al*., 1995), *Colletotrichum gloeosporioides* (Noiaium and Soytong, 1999), *Fusarium oxysporum* f. sp. *lycopersici* (Soytong *et al*., 1999a), *Phytophthora palmivora* (Pechprome and Soytong, 1996; Sodsa-art and Soytong, 1998), *P. parasitica* (Usuwan and Soytong, 1998), *Venturia inegualis* (Heye and Andrews, 1983).

The screening of *Chaetomium* species as biological control agents has been carried out in Thailand since 1989, resulting in the development of a biological formulation from *C. cupreum* CC1-10 and *C. globosum* CG1-12.

The product has now been developed into pellet and powder formulations and registered for a Patent Right No.6266, Intl. cl. [5] AO 1 N 25 / 12 in 1994 (Soytong and Soytong, 1996).

The objective of this study was to evaluate the formulated biological product of *Chaetomium* spp. As a control agent of Bud Rot and Basal Dry Rot of Bottle palms in the field.

Materials and methods

Isolation and pathogenicity test

The pathogen, *Thielaviopsis paradoxa* was isolated from a range of symptoms on the Bottle Palm including stem bleeding, bitten leaf, dry basal rot and bud rot. The disease plant parts were cut at the advanced margin of lesions into small pieces, 1-2 cm long, washed surface disinfected for 1 min in 10% sodium hypochlorite, followed by there washing in sterile distilled water and transferred onto isolating medium (potato dextrose agar (PDA), pH. 6). The mycelia growing out of the tissue was sub-cultured to PDA and isolated into pure culture.

Pathogenicity tests

The isolates were tested for pathogenicity using detached leaves in the laboratory. Agar culture discs (0.5 cm diam.) containing mycelium of the growing the pathogen were placed onto the leaf surface, incubated in a moist chamber and incubated at room temperature (27-30ºC.) for 7 days. The non-inoculated leaves treated with sterile agar discs served as controls. (Fig. 1). The experiment was repeated five times.

Bi-culture antagonistic tests

Specific strains of antagonistic fungi e.g. *Chaetomium cupreum* Ames (CC) and *C. globosum* Kunze (CG) were isolated and screened for biological control of many plant pathogens as described in previous work (Soytong, 1991; Soytong, 1995). Each isolate was individually tested for their antagonistic ability to inhibit the growth of *T. paradoxa*. Tests were carried out in laboratory using a bi-culture antagonistic method as described by Soytong (1992). Completely Randomized Design (CRD) with four replications were used for the experiment. Data collection included colony diameter (cm), number of conidia and chamydospores, and analysis of variance was statistically computed. Treatment means were compared using Duncan

Multiple Range Test (DMRT) at P = 0.05 and 0.01. The experiment was repeated two times.

Field trails

Five year old Bottle Palms naturally infected with *Thielaviopsis paradoxa* having bud rot, bitten leaf and basal rot symptoms were used in this study. The chemical fungicides benomyl, terrachor, and carbendazim had previously been used by the owner to control disease, but the pathogen may have become resistant to these fungicides; thus the palms were seriously infected and showed slow decline. These infected trees were used in the experiment and the disease incidence was determined before the experiment.

Before the treatments, the other cultural practices such as adjusting soil acidity (pH) by liming ($CaCO_3$) 1 kg/tree, adding organic compost 3 kg/tree, removing diseased plant parts and improving water drainage were carried out and the soil moisture content was periodically maintained. The pelleted formulation of *Chaetomium* was applied into the rhizosphere soil at the rate of 20 g/tree. A crude methanol extract of *C. globosum* and *C. cupreum* was also periodically sprayed every 7 days at the rate of 50 ml/ 20 litres of water. In the control experiment, the chemical fungicide, carbendazim was sprayed at the recommended rate in the same manner. Randomized Complete Block Design (RCRD) with six replications was used for the experiment. Disease levels were recorded on a scale of 1-5 and analysis of variance statistically computed. Treatment means were compared using Duncan Multiple Range Test (DMRT) at P = 0.05 and 0.01. The experiment was repeated two times.

Results and discussion

Isolation and pathogenicity test

Thielaviopsis paradoxa was isolated from rotting buds, and diseased basal stems and roots of the Bottle Palms. The isolates were confirmed to be the causative agent as they were shown to have the ability to infect detached leaves in the laboratory (Fig. 1).

Thielaviopsis paradoxa was first reported to be a pathogen of the Bottle Palm in Thailand. In this study, various symptoms were observed in the diseased plant parts, including bud rot, bitten leaf, and blackish-brown lesions developing on external and internal tissues. The new leaves were usually deformed, and reduced pinnate, and had black necrotic tips. This was similar to the symptoms reported on other palms by Chase and Broschat (1993). The

anamorph *T. paradoxa* was only isolated, and the teleomorph *Ceratocystis paradoxa* was not observed. The anamorph produced an abundance of endo-conidia and chlamydospores. The conidiophores are usually straight, hyaline to pale brown, up to 200 µm long, with a terminal conidia-bearing cells through which conidia are borne in chains. The conidia are cylindrical with square ends, hyaline to pale brown, 7-12 × 3-5 µm. The chlamydospores are borne terminally in chains from short hyphal branches, and are pale brown to brownish-black, smooth, oval and 10-20 × 5-10 µm (Fig. 2).

Results show that *T. paradoxa* causes bud rot of the Bottle Palm which Chase and Broschat (1993) reported in other palm species including *Areca catechu, Brathea edulis, Caryota* spp., *Cocos nucifera, Elaeis guineensis, Phoenix africanus, P. canariensis, Phoenix dactylifera, Rhapis* sp., *Roystonea elata, Sabal palmetto, Syagus romanzoffian,* and *Washingtonia filifera*. This pathogen has also been reported to have a wide host range outside the Arecaceae which includes many economically important hosts (Mazumder, 1995).

Fig. 1. Pathogenicity test of *Thielaviopsis paradoxa* on inoculated Bottle Palm's leaves. Left = non-inoculated detached leaf and right = inoculated leaf

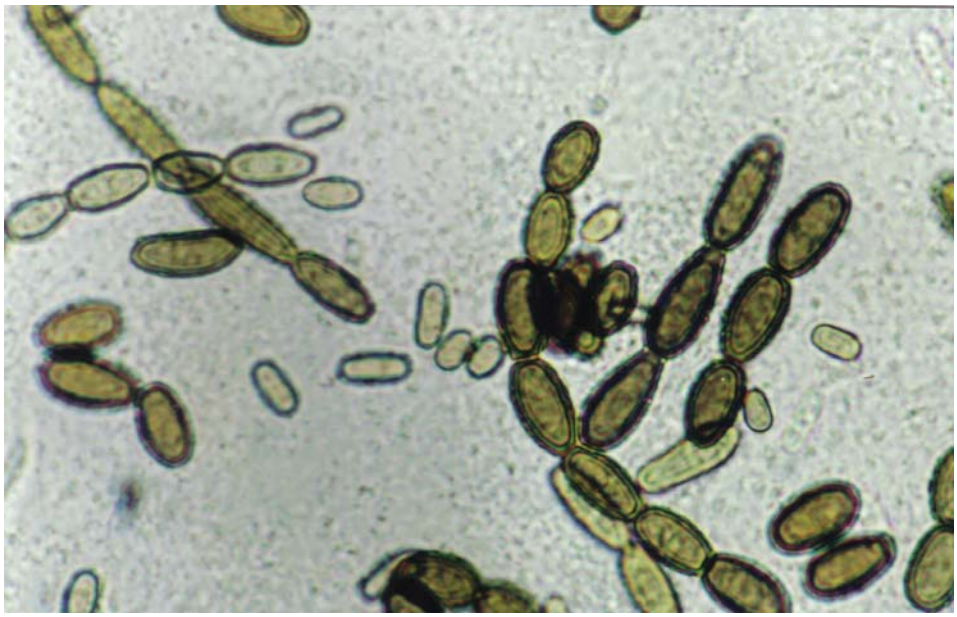

Fig. 2. Conidia and chlamydospores of *Thielaviopsis paradoxa* causing bud rot and dry basal rot of Bottle Palm (400×).

Bi-culture antagonistic tests

Specific strains of *C. cupreum* and *C. globosum* resulted in significant inhibition of colony growth, conidia and chlamydospore production of the fungal pathogen *T. paradoxa* (Tables 1-2 and Figs 3-4). Bi-culture antagonistic assays also showed that *C. cupreum* and *C. globosum* were effective antagonists to *T. paradoxa in vitro*. Kommedahl and Chang (1975) stated that antagonistic activity from the hyphae of *Chaetomium* spp. is effective only when the hyphae of test pathogens were near, implying antibiosis, as the mechanism of the biological control. In this study, it was shown that the mechanism of biological control was in terms of competitive growth; the antagonistic *Chaetomium* spp. grew over the colony of *T. paradoxa* over time.

Fig. 3. Bi-culture antagonistic test between *Chaetomium globosum* and *Thielaviopsis paradoxa*.

Fig. 4. Bi-culture antagonistic test between *Chaetomium cupreum* and *Thielaviopsis paradoxa*.

Table 1. Bi-culture antagonistic test between *Thielaviopsis paradoxa* and antagonistic fungi.

Fungi	*Thielaviopsis paradoxa*		
	Colony (cm)	**Conidia (× 10^6 ml^{-1})**	**Chlamydospores (× 10^6 ml^{-1})**
Ch. cupreum	4.28b[1/]	22.5b	21.3b
Ch. globosum	3.58c	22.2b	21.4b
T. paradoxa	9a	44.4a	38.1a
C.V. (%)	2.85	13.07	10.91

[1/] Average of six replications. Means followed by the same letter in a column were not significantly different by DMRT at P = 0.01.

Table 2. Percentage inhibition of growth, conidia and chlamydospores of *Thielaviopsis paradoxa* in the bi-culture antagonistic test.

Fungi	*Thielaviopsis paradoxa*		
	Colony	**Conidia**	**Chlamydospores**
Ch. cupreum	52.44[1/]	46.64	43.96
Ch. globosum	60.18	49.58	43.66

[1/] Mean of six replications.

Field trails

Bottle Palms seriously infected in the field with Bud Rot and Basal Dry Rot caused by *Thielaviopsis paradoxa* recovered after applying *Chaetomium* biological products integrated with the other disease control measures. The *Chaetomium* biological product significantly reduced the disease incidence by 75% within 30 days after application when compared with the controls (Table 3 and Fig. 5). These results are similar to those found in previous work on biological control of *Phytophthora* rot of durian in the field which demonstrated that the *Phytophthora* pathogen could be reduced after applying *Chaetomium* into infested soil (Prechprome and Soytong, 1997). The amount of pathogen inocula in soils treated with *Chaetomium* is reduced due to antagonistic activity. Kohl *et al.* (1995) found *C. globosum* has high competitive ability which is a prerequisite for successful saprobic antagonists introduced to senescing or necrotic leaf tissue under field conditions. They concluded that *Chaetomium* spp. are strongly competitive and have the ability to colonize organic compost and suppress pathogens in the soil. Similar results were found in previous work which demonstrated that the application of *Chaetomium* biological products reduced the pathogen inoculum and disease incidence of *Phytophthora* rot of Sweet Orange (Usuwan and Soytong, 1998) and *Phytophthora* rot of black pepper (Sodsa-ard and Soytong, 1999). In the

Fig. 5. Field evaluation of *Chaetomium*-biological fungicide to control *Thielaviopsis* Bud Rot and Dry Basal Rot of Bottle Plam at Nong Nuch Tropical Garden, Thailand. (a. before treatment, b. treated tree after 30 days, c. treated tree after 45 days and d. treated tree after 60 days).

present study it was shown that *Chaetomium* biological products could successfully control Bud Rot and Basal Stem Rot of Bottle Palms in the field.

Table 3. Application of biological products of *Chaetomium* to control Bud Rot and Basal Dry Rot of Bottle Palm in infested field-soil.

Treatments	Disease level[1/]		Disease reduction (%)[2/]
	Before	After	
Chaetomium	4.0 a[3/]	1.0 b	75
Control	4.0 a	5.0 a	---

[1/] Disease level, 1 = healthy plant (0%), 2 = rotting bud and basal stem 1-25%, 3 = rotting bud and basal stem 26-50%, 4 = rotting bud and basal stem 51-75% and 5 = rotting bud and basal stem 76-100% (plant died).
[2/] Disease reduction (%) = disease level in control trees - disease level in treated trees / disease level in control trees × 100.
[3/] Average of six replications. Means followed by a common letter in a column were not significantly different by DMRT at P = 0.01.

Acknowledgements

The authors wish to acknowledge the Nong Nuch Tropical Garden, Pattaya, Thailand, for funding the research project.

References

Chase, A.R. and Brosshat, T.K. (1993). *Diseases and Disorders of Ornamental Palms.* APS Press. USA.

Dransfield, J. (1996). *Hyophobe lagenicaulis*. Curtis's Botanical Magazine 13: 186-190.

Gariani, N.K., Nuesery, S.M. and Edongali, E.A. (1994). Disease and pest outbreaks. Libya. Black scorch disease of date palms (*Phoenix dactylifera*) in Libya. Arab and Near East Plant Protection Newsletter 19: 40.

Heye, C.C. and Andrews, J.H. (1983). Antagonism of *Athelia bombucina* and *Chaetomium globosum* to the apple scab pathogen, *Venturia inequalis*. Phytopathology 76: 650-654.

Jones, D. (1995). *Palms Throughout the World*. Smithsonical Institution Press. USA.

Kohl, J., Molhoek, W.M.L., Van Der Plas, C.H. and Fokkema, N.J. (1995). Effect of *Ulocladium atrum* and other antagonistics on sporulation of *Botrytis cinerea* on dead lily leaves to field conditions. Phytopathology 85: 393-401.

Kommedahl, T. and Chang, M.I. (1975). Biocontrol of corn root infection in the field by seed treatment with antagonistics. Phytopathology 65: 296-300.

Mazumder, N. (1995). Reaction of coconut cultivars and hybrids to the incidence of different pests and diseases, Horticultural Journal 8: 147-149.

Mubararak, H.F., Riaz, M., Saeed, A.S. and Hameed, J.A. (1994). Physiological studies and chemical control of black scorch disease of date palm caused by *Thielaviopsis* (= *Ceratocystis*) *paradoxa* in Kuwait. Pakistan Journal of Phytopathology 6: 7-12.

Noiaium, S. and Soytong, K. (1997). Integrated biological control of mango var. Choakanon. Proceedings of the Sixth International Mango Symposium. Pattaya, Thailand: 1-13.

Prechaprome, S. and Soytong, K. (1997). Integrated biological control of durian stem and root rot caused by *Phytophthora palmivora*. Proceedings of the First International Symposium on Biopesticides. Thailand, October 27-31, 1996: 228-237.

Sodsa-art, P. and Soytong, K. (1999). Biological control of black pepper root and basal stem rot in the field. Proceedings of Symposium on Biological Control in the Tropics, MARDI Training Centre, Malaysia, 18-19 March 1999: 68-70.

Soytong, K. (1991). Species of *Chaetomium* in Thailand and screening for their biocontrol properties against plant pathogens. Proceedings of the XII International Plant Protection Congress. 11-16 August 1991, Rio de Janeiro, Brazil, supported by IFS, Sweden.

Soytong, K. (1992). Biological control of rice blast disease by seed coating with antagonistic fungi. Songklanakarin Journal of Science and Technology 14: 59-65.

Soytong, K. (1995). *Chaetomium* as a biocontrol agent against plant pathogens. Proceedings of the XIII International Plant Protection Congress, 2-7 July 1995, The Hague-The Netherlands.

Soytong, K. and Soytong, K. (1996). *Chaetomium* as a new broad spectrum mycofungicide. Proceedings of the First International Symposium on Biopesticides, Thailand, October 27-31, 1996: 124-132.

Soytong, K., Jindawong, N. and Yang, Q. (1999). Evaluation of *Chaetomium* for biological control of *Fusarium* wilt of tomato in P.R. China. Proceedings of the 5th International Conference on Plant Protection in the Tropics, 15-18 March 1999, Malaysia: 484-487.

Soytong, K., Usuwan, P., Kamokmedhakul, S., Kanokmedhakul, K., Kukongviriyapan, V. and Isobe, M. (1999). Integrated biological control of *Phytophthora* rot of sweet orange using mycofungicides in Thailand. Proceedings of the 5th International Conference on Plant Protection in the Tropics, 15-18 March 1999, Malaysia: 329-331.

Usuwan, P. and Soytong, K. (1998). Biological control of Phytophthora rot of citrus in the field. Proceedings of the 24th Congress on Science and Technology, 19-21 October 1998, Thailand.

(Received 27 September 2005; accepted 5 December 2005)