

Nano-particle derived from *Chaetomium cupreum* CC3003 against Anthracnose of coffee var. Arabica

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Abstract

Colletotrichum gloeosporioides is proved to be a pathogenic isolate causing anthracnose disease on coffee var. Arabica in Lao PDR. *Chaetomium cupreum* CC3003 inhibits sporulation of *C. gloeosporioides* by 42.60 % in 30 days. The tested nano CCH, nano CCE and nano CCM derived from *C. cupreum* CC3003 significantly inhibits *C. gloeosporioides* that cause coffee anthracnose at low concentrations of about 3-15 ppm. The tested nano-particles applied to inoculated coffee seedlings significantly reduce coffee anthracnose. Research and development on nano-particles extracted from fungi are necessary to discover new strategies to control plant disease.

Introduction

The Lao People's Democratic Republic is located in Southeast Asia. The country predominantly depends on rural economy, with about 83% of the population living in the rural areas and some 66% relying on subsistence agriculture *MAF*.¹ Economically, coffee is one of the most important crops that resulted from the horticultural skills of the Dutch.² Coffee is distributed to many places around the world.³ As a result, coffee is now one of the most important exported commodities from approximately 50 countries in Latin America, Africa, Asia and Lao PDR.^{4,5} Coffee anthracnose caused by *Colletotrichum gloeosporioides* was confirmed by molecular phylogeny that invaded coffee beans and leading to economic

damage of coffee plantations in Lao PDR. Crude extracts from *Chaetomium cupreum* CC3003 inhibited *C. gloeosporioides*. The bio-formulation of *C. cupreum* decreased the incidence of anthracnose.⁶ In recent years, nanotechnology has started to investigate plant disease control measures for food safety concerns in agriculture.⁷ With increasing nanotechnology for plant disease protections that have been developed by some researchers, nano-particles from natural products may become the new approach for plant disease control. Nanotechnology in crop production is to build up the materials at the molecular level into nano particles.⁸ Some scientists have been investigating organic nanomaterials and different kinds of nano-particles for biological properties.^{9,10} Nano formulations have been started to produce from natural products to control insects.⁴ Bioactive compounds from natural products derived from *C. cupreum* CC3003 were investigated to be active against several plant pathogens.¹¹ The objective of the research was to study the nano-particles from *C. cupreum* CC3003 to control anthracnose of coffee var. Arabica.

Materials and Methods

Culture of pathogen and *Chaetomium cupreum* CC3003

Infected Coffee fruits by anthracnose of the Arabica variety was collected from Paksong Highland, Champasak Province, Lao PDR. Pure cultures of *Colletotrichum gloeosporioides* and *Chaetomium cupreum* CC3003 were isolated from both leaves and fruits and results were confirmed using molecular phylogenetic.⁶ They were separately transferred onto potato dextrose agar (PDA), incubated at temperature between 27-35°C for 15 days. Morphological developments were recorded and photographed.

Pathogenicity tests

Pathogenicity tests were done by detached leaf method. The agar plug of pathogen was inoculated into wounded leaf and kept in a moist chamber. An agar plug of PDA alone onto wounded leaf was served as a control. Lesion size (mm) was recorded. The experiment was repeated four times.

Dual culture test

Chaetomium cupreum CC3003 produce antibiotic substances used in this study as reported by Kanokmedhakul *et al.*¹² An agar plug of 0.5 cm with mycelia of *C. cupreum* CC3003 was moved to one side of PDA plate and the other agar plug of

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pathogen was placed in the opposite side at an equal distance from each other in the tested plates. Control plates were cultivated either with pathogen or with *C. cupreum* CC3003 alone. The colony diameter (cm) and number of pathogen spores in dual culture plate and control plate were counted. The experiment was performed using CRD (Completely Randomized Design), and compared treatment means using DMRT (Duncan's multiple range test). It was repeated four times.

Effect of nano-particles derived from *Chaetomium cupreum* CC3003 against *Colletotrichum gloeosporioides* in 7 days

The nano-particles used in this research finding derived from *C. cupreum* CC3003 were 171 nanometers in average.¹³ The experiment was performed using a Completely Randomized Design (CRD)

with 2 factors. Different kinds of nano-particles, nano-CCH, nano-CCe and nano-CCM were investigated with different concentrations of 0, 3, 5, 10 and 15 ppm. The experiment was repeated four times. Colony diameter and number of spores were collected and a computer analysis of variance was performed, then compared with Duncan Multiple Range Test (DMRT). The effective dose at 50% was calculated using probit analysis program. The normal and abnormal spores from each treatment were also recorded.

Evaluation of bioformulation produced from spores of *Chaetomium cupreum* CC3003 and nano-particles to control anthracnose of coffee var *Arabica* in pot experiment

The six months seedlings of coffee var *Arabica* was used to inoculate *C. gloeosporioides* on wounded leaves at a concentration of 1×10^6 spores/mL, each wound inoculated with 1 mL. *Chaetomium*-bioformulation was prepared according to the method of Soyong *et al.*¹¹ and applied at the rates of 10 g and 20 g/20 water and nano-particles 15 ppm., then the wounded lesions were sprayed after inoculation for 24 h. Lesion size was recorded and calculated using ANOVA and DMRT comparison tests. The experiment was tested using RCBD and repeated four times.

Results

Culture of pathogen

C. gloeosporioides causing anthracnose of coffee was cultured on PDA for 15 days



Figure 1. Culture of *Colletotrichum gloeosporioides* causing anthracnose of coffee on PDA for 15 days, right = pure culture and left = conidia.

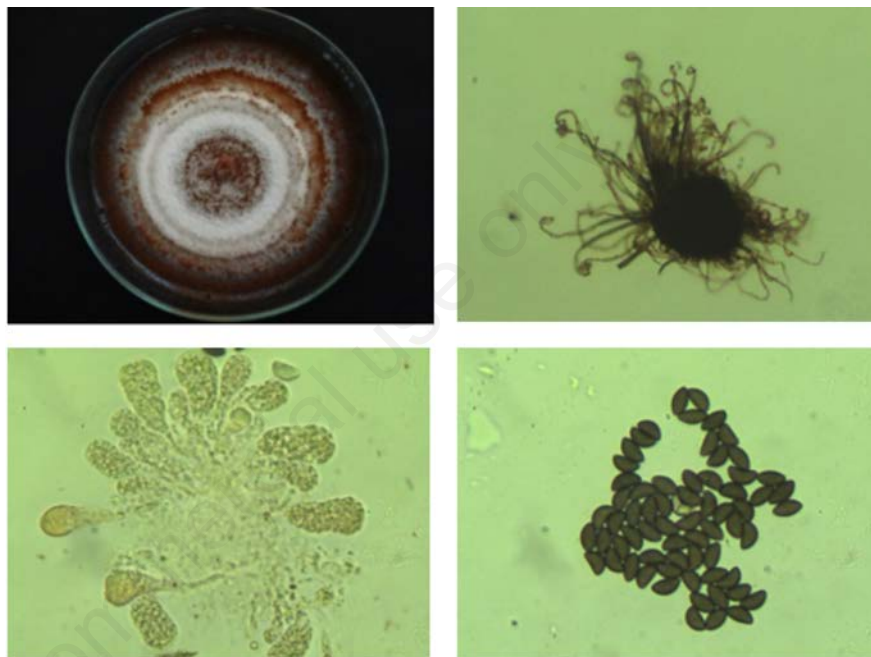


Figure 2. Culture of *Chaetomium cupreum* CC3003 on PDA for 3 weeks, upper right = pure culture, upper left = perithecium, lower right = asci and lower left = ascospores.

Table 1. Nano-particles derived from *Chaetomium cupreum* CC3003 against *Colletotrichum gloeosporioides* in 7 days.

Nano-particles	Conc.	Colony diameter (cm)*	Number of spores ($\times 10^6$)	Colony* inhibition	Spore* inhibition	ED ₅₀ (ppm)
Nano CCH	0	5.00a ¹	12.23bc	0.00f	0.00f	40.42
	3	4.85ab	8.63de	3.10ef	28.24e	
	5	4.76bc	6.63ef	4.85de	52.00cd	
	10	4.49d	2.13gh	10.25c	82.27ab	
	15	3.83e	2.13gh	23.34b	82.42ab	
Nano CCE	0	5.00a	13.88ab	0.00f	0.00f	37.29
	3	4.93ab	9.88cd	1.45ef	28.45e	
	5	4.80bc	6.63ef	4.00de	52.00cd	
	10	4.64c	4.25fg	7.10d	69.35bc	
	15	3.79e	0.88h	24.09b	93.57a	
Nano CCM	0	5.00a	15.88a	0.00f	0.00f	10.90
	3	4.92ab	7.75de	1.50ef	49.66d	
	5	4.80bc	6.38ef	3.95de	58.77cd	
	10	4.35d	3.25gh	13.00c	79.92ab	
	15	3.32f	2.50gh	33.75a	83.59ab	
C.V. (%)	1.79%	18.93%	18.75%	18.47%		

Means of four replication which followed by a common letters are not significantly different by DMRT at $P=0.05$. *Inhibition (%) = $(R1-R2)/R1 \times 100$ where R1 = colony or spores of pathogen in control, R2 = colony or spores in treatment.

and the observed colony had a greyish white colour, and a single cell of conidia (Figure 1).

Chaetomium cupreum CC3003

The isolate was cultured on PDA for 3 weeks to produce perithecia, asci and ascospores (Figure 2).

Pathogenicity test

A pathogenicity test was completed to reconfirm the pathogenic isolate of Arabica coffee, which was observed in 15 days after inoculation. The inoculated lesions showed the pathogen-infected lesions with an average of 2.17 cm when compared to the control that shows no symptoms as you can observe in the Figure 3.

Dual culture test

A dual culture test was done between effective antagonistic fungus, *C. cupreum* CC3003 and pathogenic isolate, *C. gloeosporioides* showed that in the dual culture plates the colony diameter of pathogen was 9.00 cm in average, while in dual culture plate it was 6.28 cm in average and has a 30% of colony inhibition. It was interesting that in the dual culture plate the average number of pathogen spores was 15.13×10^6 and in the control plate the average was 26.25×10^6 spores, and the inhibition percentage was 42.60% after incubation for 30 days (Figure 4).

Effect of nano-particles derived from *Chaetomium cupreum* CC3003 against *Colletotrichum gloeosporioides*

Results showed that at the concentration of 15 ppm, nano CCM showed a significant lower colony growth (3.32 cm) than nano CCE and nano CCH. The colony diameters were respectively 3.79 and 3.83 cm when compared to the non-treated control (5 cm). Moreover, at the concentration of 15 ppm, nano CCE showed a significant lower spore number (0.88×10^6) than nano CCH and nano CM, which were respectively 2.13×10^6 and 2.5×10^6 spores (Table 1). Nano CCM showed the highest colony inhibition of 33.75%, and followed by nano CCE and nano CCH that were respectively 24.09% and 23.34%. However, nano CCE showed a significantly high inhibition of sporulation of 93.57%, and followed by nano CCM, which were 83.59% and nano CCH that were 82.24%, respectively. The results concluded that nano CCE expressed the highest antifungal activity against *Colletotrichum gloeosporioides* at the ED_{50} of 10.09 ppm, and followed by nano CCE and nano CCH which the ED_{50} were 37.29

and 40.42 ppm, respectively. All tested nano-particles derived from *Chaetomium cupreum* CC3003, nano CCH, nano CCE and nano CCM at concentrations of 3, 5, 10, and 15 ppm, actively expressed the control mechanism of antibiosis that the pathogen cells were abnormal and showed a possible loss of pathogenicity (Figure 5).

Evaluation of bio-formulation produced from spores of *Chaetomium cupreum* CC3003 and nano-particles to control anthracnose of coffee var. Arabica in pot experiment

Results showed that coffee seedlings inoculated with *C. gloeosporioides* treated

with nano-particles for six months showed a decrease of 35.40% in terms of disease incidence. While the one treated with *Chaetomium*-bioformulation 20 g/20 L of water and *Chaetomium*-bioformulation 10 g/20 L of water showed a disease reduction of 0.83% and 1.13%, respectively, after treatment for 30 days in a pot experiment (Table 2).

Discussion and Conclusions

Anthracnose of coffee var. Arabica proved to be caused by *C. gloeosporioides* as previously reported by Vilavong *et al.*⁶



Figure 3. Pathogenicity test of *C. gloeosporioides* causing anthracnose of coffee, upper = non-inoculated control and lower = inoculated with pathogenic isolate.



Figure 4. Dual culture test between *C. cupreum* CC3003 and *C. gloeosporioides*, right = *C. cupreum* CC3003, middle = dual cultures and left = *C. gloeosporioides*.

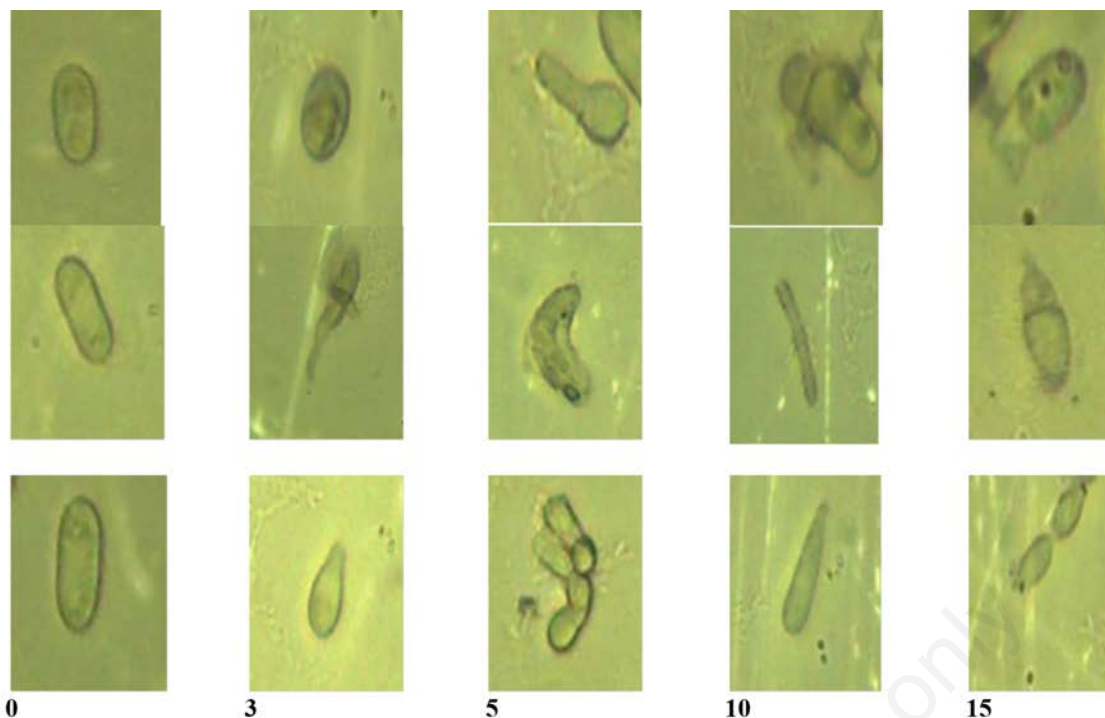


Figure 5. Abnormal conidia after treated with Nano CCH, nano CCE and nano CCM.

Table 2. Symptoms and disease reduction after treated with bioformulation and nano-particles derived from *Chaetomium cupreum* CC3003 in 30 days.

Treatments	Lesions (mm)	Disease reduction*
Inoculated	1.45a ¹	----
Chaetomium-bioformulation 10 g/20 L of water	1.13b	28.31
Chaetomium-bioformulation 20 g/20 L of water	0.83ab	26.55
Nano-particles 15 ppm	0.73a	35.40
C.V. (%)	78.62%	----

Means of four replications which followed by a common letters are not significantly different by DMRT at $P=0.05$. *Disease reduction (%) = Lesion size in inoculated control – Lesion size in treatment/ Lesion size in inoculated control $\times 100$.

and the pathogenic isolate was confirmed by molecular phylogeny. The isolate used of *C. cupreum* CC3003 was effective as Kanokmedhakul *et al.*¹² reported that it released (+) Rotiorin the active compound against *Candida albican* at IC50 of 0.6 ppm. It is possible that *C. cupreum* CC3003 inhibits sporulation of *C. gloeosporioides* by 42.60% within 30 days in dual culture test. The research finding was similar to the results of Vilavong *et al.*⁶ As a result, the nano-particles of *Chaetomium cupreum* CC3003, nano CCH, nano CCE and nano CCM inhibits *C. gloeosporioides* causing coffee anthracnose at concentration of 3-15 ppm. at concentrations of 3, 5, 10, and 15 ppm, similar results found from Tann *et al.*¹⁴ who used these nano-particles as a treatment to control *Curvularia lunata* that

cause leaf blight of rice in Cambodia.

The research findings of using nano-particles to treat coffee seedlings inoculated with *C. gloeosporioides* reduced anthracnose disease of coffee. Tann *et al.*¹⁴ reported that bio-formulation of *C. cupreum* and nano product from *C. cupreum* showed a good control of blight disease in the rice pot experiment. Bio-formulation of *C. cupreum*, and nano products from *C. cupreum* reduced the incidence of disease by 58.33%. The use of bioactive compounds from *Chaetomium* species were proved to be an effective antifungal against plant pathogens.¹¹ Research on nano-particles loaded with bioactive substances from effective fungi will need more investigation and development to ensure safe crop production.

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