Chapter

Chaetomium Application in Agriculture

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Abstract

Chaetomium species for plant disease control are reported to be antagonize many plant pathogens. It is a new broad spectrum biological fungicide from *Chaetomium* species which firstly discovered and patented No. 6266, International Code: AO 1 N 25/12, and registered as Ketomium® mycofungicide for plant disease control in Thailand, Laos, Vietnam, Cambodia and China. *Chaetoimum* biofungicide and biostimulants are applied to implement integrated plant disease control. It showed protective and curative effects in controlling plant disease and promoting plant growth. It has been successfully applied to the infested soils with integrated cultural control for the long-term protection against rice blast (*Magnaporte oryzae*), durian and black Pepper rot *(Piper nigram* L.) (*Phytophthora palmivora*), citrus rot (*Phytophthora parasitica*) and strawberry rot *(Fragaria* spp.) caused by *Phytophthora cactorum,* wilt of tomato (*Fusarium oxysporum* f. sp. *lycopersici*), basal rot of corn *(Sclerotium rolfsii*) and anthracnose (*Colletotrichum* spp.) etc. Further research is reported on the other bioactive compounds from active strains of *Chaetomium* spp. We have discovered various new compounds from *Ch. globosum*, *Ch. cupreum*, *Ch. elatum*, *Ch. cochliodes*, *Ch. brasiliense*, *Ch. lucknowense*, *Ch. longirostre* and *Ch. siamense.* These new compounds are not only inhibiting human pathogens (anti-malaria, anti-tuberculosis, anti-cancer cell lines and anti-*C. albicans* etc) but also plant pathogens as well. These active natural products from different strains of *Chaetomium* spp. are further developed to be biodegradable nanoparticles from active metabolites as a new discovery of scientific investigation which used to induce plant immunity, namely microbial degradable nano-elicitors for inducing immunity through phytoalexin production in plants e.g. inducing tomato to produce alpha-tomaline against *Fusarium* wilt of tomato, capsidiol against chili anthracnose, sakuranitin and oryzalexin B against rice blast, scopletin and anthrocyaidin against *Phytophthora* or *Pythium* rot Durian and scoparone against *Phytophthora* or *Pythium* rot of citrus. *Chaetomium* biofungicide can be applied instead of toxic chemical fungicides to control plant diseases.

Keywords: *Chaetomium*, Biofungicides, plant diseases, plant immunity, phytoalexin

1. Introduction

Chaetomium Kunze belongs to Ascomycota of the *Chaetomiaceae* which established by Kunze in [1] and it is one of the largest genera of saprophytic ascomycetes which comprise more than 300 species worldwide [2–6]. *Chaetomium* is recorded to be a potent antagonists of various plant pathogens, especially soil-borne and seed borne pathogens [4, 7–9]. *Chaetomium* is also reported to be antagonistic to the growth of bacteria and fungi through competition for nutrients, mycoparasitism, antibiosis, or various combinations [10]. The application of *Chaetomium* as a antagonist to control plant pathogens was first reported in 1954 by Tveit and Moore who found *Ch. globosum* and *Ch. cochliodes* grown on surface of oat seeds provided to control *Helminthosporium victoriae* [11]. *Chaetomium globosum* and *Ch. cochlioides* noted to inhibit the growth of *Fusarium* spp. and *Helminthosporium* spp. [11]. The living spore of Ch. *globosum* significantly controlled the apple scab (*Venturia inequalis)* [12]. *Ch. globosum* produces metabolites inhibiting the growth of *P. ultimum* causing damping-off of sugar beet [13], *Rhizoctonia solani* [14], leafblight of brassicas causing *Alternaria brassicicola* [15] and can reduce the pathogenic inoculum of *Botrytis cinerea* causing deadly lily leaves in the field [16]. *Chaetomium cupreum* is reported to control soybean plant pathogens e.g. *Phomopsis* and *Colletotrichum* spp. [17]. It is reported that isolate of *Ch. globosum* produce antibiotic substances that suppressed the damping – off of sugar beet cause by *P. ultimum* [13]. *Chaetomium globosum* is noted to be a strong cellulose decomposer [18] and showed an effective antagonist of various soil microorganisms [7, 8]. Moreover, *Chaetomium* spp. are recorded to produce their active metabolites for biological activities. Several types of pure compounds are found from *Chaetomium* species e.g. benzoquinone derivatives [19].

In Thailand, *Chaetomium* spp. were studied and screened for abilities as antagonistic effect againist phytopathogens in 1989 by Soytong *et al*. [20]. *Chaetomium cupreum* and *Ch. globosum* were reported to decrease leaf spot disease of corn caused by *Curvularia lunata*, rice blast caused by *Pyricularia oryzae* and sheath blight of rice caused by *Rhizoctonia oryzae* [21, 22]. *Chaetomium* spp. are also strongly recommended in competitive and high ability to make organic compost and to suppress pathogen in the soil. *Chaetomium* spp. are known to be the strictly saprophytic coprophilousn. It can be found in organic materials, plant debris and high organic soil [23]. Some species produce cellulase to decompose cellulose, lignin and other organic materials and serve as a biological control agent against several phytopathogens [20].

Chaetomium cupreum and *Ch. globosum* are noted to decrease leaf spot of corn (*Curvularia lunata*), rice blast (*Pyricularia oryzae*), sheath blight (*Rhizoctonia oryzae*) and tomato wilt (*Fusarium oxysporum* f.sp. *lycopersici*) [22, 24]. Chetomanone, ergosterol, ergosteryl palmitate, chrysophanol, chaetoglobosin C, alternariol monomethyl ether, echinuline and isochaetoglobosin D were produced from *Ch. globosum* KMITL-N0802 [25] which implies antibiosis. *Chaetomium cupreum* was significantly reduced the growth of seed-borne pathogen of rice e.g. *Curvularia lunata, Drechslera oryzae, Fusarium moniliforme* and *Pyricularia oryzae* [26]. *Chaetomium globosum* was exprsssed significantly suppression to tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* and *Pseudomonas solanacearum in Thailand* [23, 27] while *Ch. cupreum* reported to give a good control tomato wilt in the fields [28]. The specific effective strains of *Chaetomium* spp. have been formulated as biological products in the forms of pellet and powder [29] that could effectively control many soil borne plant pathogens. The *Chaetomium* bioproducts were reported to have a good potential in control of *Thielaviopsis* Bud Rot of Bottle palm caused by *Hyophorbe lagenicaulis* in the fields [30]. *Chaetomium globosum* and *Ch. cupreum* are successfully applied to control root rot disease of citrus, black pepper, strawberry and reduce damping off disease of sugar beet [20, 31]. The powder and pelletized formulations of Ketomium®, as a broad spectrum biofungicides has been registered as a biological biofertilizer for degrading organic matter and to induce plant immunity and stimulate plant growth [20]. It was recorded that Ketomium® mycofungicide from Thailand was the most efficient to control raspberry spur blight (*Didymella applanate*) and reduced potato disease (*Rhizoctonia solani*) and increasing potato yield [32]. After 2 years in storage, Ketomium® biofungicide from Thailand was still capable to inhibit the growth of plant pathogens in higher doses [31].

The biofungicide produced from *Ch. globosum* and *Ch. cuprem* resulted in controlling *Phytophthora* root rot of durian (*Phytophthora palmivora*) in the fields which the *Phytophthora* pathogen reduced after applying *Chaetomium* into soils planted Durian var. Monthong in Thailand [33]. *Chaetomium* biofungicide is also reported to control *Phytophthora parasitica* in fields planted to Citrus in the most serious disease area in Thailand [34]. Soytong *et al.* [20] stated that Ketomium® was applied to *Fusarium*-infested soils where tomatoes were grown and it had sucessfully controlled the pathogen inoculum and reduced disease prevalance. Ketomium® have been successfully controlled as *Fusarium*-suppressive soils. The tomato plants treated with Ketomium® and pentachloronitrobenzen (PCNB) completely controlled *F. oxysporum* f. sp. *lycopersici* (tomato wilt). *P. palmivora* (black pepper rot). It is penciled that the tested Ketomium® biological product has been proved to control several diseases in the fields to control root rot of durian [33], black paper [35] and citrus [36]. Further research has done to formulate those active antagonistic *Chaetomium* species to be biological fungicide to control plant diseases e.g. late blight of potato, citrus rot disease, white rot disease of para rubber. It is also reported that the specific strains of *Ch. globosum* can be inhibited root knot nematode and insect control.

2. Biological control of potato late blight using *Chaetomium* biofungicide

Application of *Chaetomium*-biofungicide is sucessfully controlled late blight late blight of potato caused by *Phytophthora infestans* in northern Thailand. Before experiment, It was found that *P. infestans* spread directly to the potato plants in the field through zoosporangia and resulted in a serious diseaase and lost of yield. It was investigated that *P. infestans* found in the soil planted with potato in high pathogen inocula before experiment (**Figure 1**). Ketomium®-biofungicide decreased the disease incidence in the infested fields when compared with the non-treated

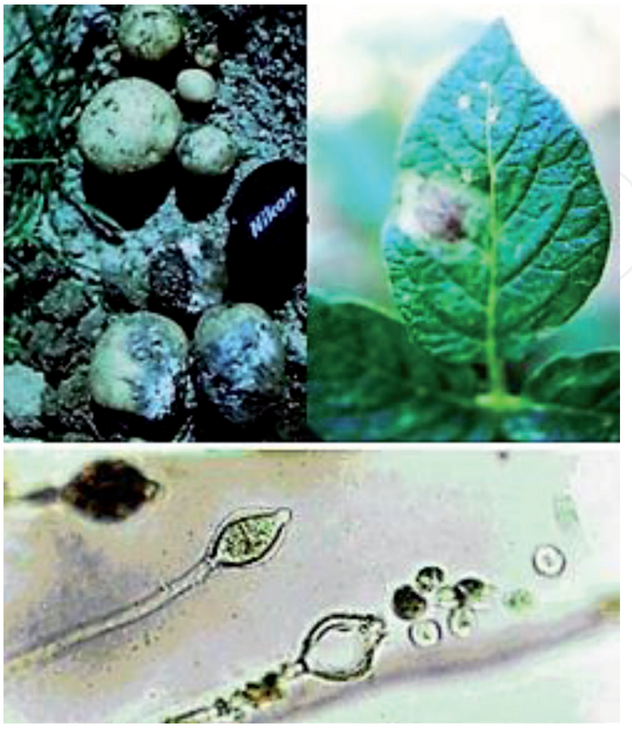

Figure 1.
Symptoms of late blight caused by Phytophthora infestans *on leaves (upper left) and dry rot on potato tuber (upper right). Sporangiophore, sporangium of* Phytophthora infestans *(lower part).*

Figure 2.
Experimental plot testing Chaetomium *biofungicide against the late blight of potato.*

control and the difference between *Chaetomium*-biofungicide and chemical pesticide treated fields were not significantly differed. The field experiment was carried out in Chiang Mai province, Thailand, where the soil was seriously infested with *P*. *infestan* and wherein disease previously destroyed the potato plants and resulted in total loss of the yield. Ketomium® biofungicide reduced late blight incidence by 38% as seen in **Figure 2** [37].

3. The control of citrus rot disease with *Chaetomium* biofungicide in Cambodia

P. ultimum was firstly recorded by our investigation to cause citrus (*Citrus reticula* Blanco) root rot in Cambodia. The experimental field was carried out in 1 ha of 200 trees of citrus orchard in Battambang province where the infested soil with *P. ultimum* causing root rot disease. It was observed before experiment that *P. ultimum* infected the citrus trees over 90%, and mostly citrus trees were completely destroyed which can be seen the sign of yellowing and small leaves, dieback and root rot. The four-year-old citrus trees were selected for experiment with the same disease level.

The pathogen causes a serious damage almost everywhere planted to citrus in Battambang province. *P. ultimum* infects citrus plants starting from seedlings which show yellow leaves, die back, stem rot, root rot and die. The infected citrus trees slowly decline from the second year planting and gradullay died which starting from six to seven years old, even using the chemical fungicides. The reserch found that the detached leave method was proved that only three days inoculation of *P. ultimum*, the citrus leaves turned to pale yellow and completely dark brown. The re-isolation the aggressive pathogen from lesion confirmed its pathogenicity. In field expriment, chemical fungicide, *Chaetomium* and *Trichoderma* treamenrts were compared and periodically applied every month to four-year old citrus trees in one year. All treatments were sprayed above plants and to rhizosphere soil every month as metalaxyl-10 g/20 L of water in combination with chemical fertilizers, *Chaetomium* treatment (20 g/20 L of water) and *Trichoderma* treatment (20 g/20 L of water). It was noted that all treated citrus trees significantly recovered within 3–4 months of applications. As it was seen new leave flashes and roots emerged, then the citrus trees were recovered from rot root diseaase. The biological products of either *Chaetomium* or *Trichoderma* showed significant disease control along with the metalaxyl chemical fungicide [38].

4. Application of *Chaetomium* biofungicide to control white root disease of Para rubber trees caused by Rigidoporus microporus

White root disease caused by *Rigidoporus microporus* (**Figures 3** and **4**) that was controlled by *Ch. bostrychodes* BN08, *Ch. cupreum* RY202 which inhibited the

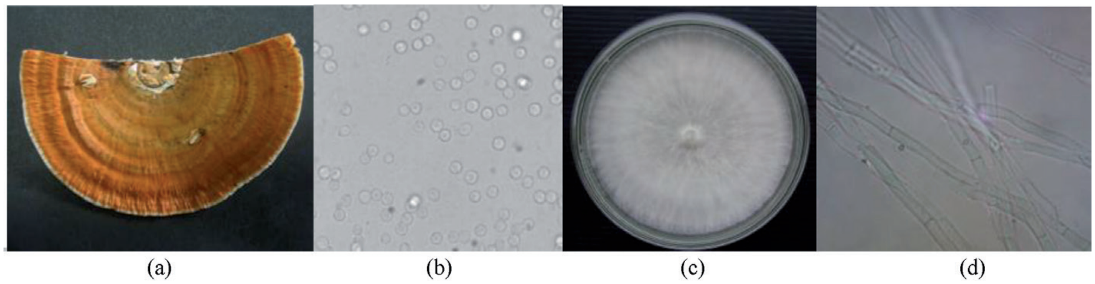

Figure 3.
Rigidoporus microporus*: Colony on PDA at 6 days (a), hypha (b) fruiting body (c), and basidiospores (d).*

Figure 4.
White root disease of Para rubber tree caused by Rigidoporus microporus*.*

growth of pathogen over 50%. *Chaetomium bostrychodes* BN08 and *Ch. cupreum* RY202 were grown over the colony of *R. microporus* within 30 days. The crude extracts from *Ch. cupreum* RY202 showed the best growth inhibition of *R. microporus* with ED_{50} values of 170, 402, and 1,220 μg/L, respectively. Rotiorinol is a bioactive compound produced from *Ch. cupreum* inhibited the growth of *R. microporus* which the ED_{50} value of 26 μg/L. The bioformulation of *Ch. cupreum* RY202 in the powder and oil form significantly inhibited *R. microporus* to infect the root of the rubber trees as reported by Kaewchai and Soytong [39]. *Chaetomium cupreum* RY202 formulated as biofungicide to control white root disease of para rubber was resulted to disease reduction in the treatments of *Ch. cupreum* RY202 in the powder form and oil form of 75%. This is the first report of our study using *Ch. bostrychodes* and *Ch. cupreum* to control the white root disease of para rubber.

5. Application of *Chaetomium* antagonistic fungi to plant parasitic nematodes and insect control

Diverse research was on *Chaetomium* which carried out for agricultural purpose. Some reserchers demonstrated the endophytic *Chaetomium* exhibited to antibitosis against nematodes e.g. root-knot nematode (*Meloidogyne incognita*) and hatch of soybean cyst nematode (*H. glycines*) and *Globodera pallida* (cyst nematode) or insects e.g. cotton aphids (*A. gossypii*) and beet armyworms (*Spodoptera exigua*).

In [40], Nitao *et al*. reported that the culture broths from *Ch. globosum* inhibited the egg hatch and juvenile mobility of root-knot nematode (*Meloidogyne incognita*) and also inhibited the hatching of soybean cyst nematode (*H. glycines*). It demonstrated that avipin is an active low molecular weight compound responding for antoganistic activity. But the testing on Muskmelon (*C. melo*) plants in steamed and unsteamed soil were inoculated with root-knot nematodes and different concentrations of avipin were not clearly shown. Zhou *et al.* [41] recorded that *Ch. globosum* TAMU 520, is endophyte from cotton (*G. hirsutum*) and systemically colonize the cotton plants through seed treatment. The endophytic *Ch. globosum* suppressed the infection of root-knot nematode (*Meloidogyne incognita*) which reduced female reproduction.

In 2012, *Ch. globosum* with nematicidal activity, metabolite and application is patented in China as a patent No. CN102925369A in 2012 by a group of Chinese Scientists which claimed that *Ch. globosum* NK106 (CGMCC6716) is preserved number. Chaetoglobosin A prevents the plant parasitic nematode due to its high toxicity and nematicidal activity against the juvenile stage of *Meloidogyne incognita*. Chaetoglobosin A at 300 mg/mL showed the lethality is 90%, decreased the nematode eggs by 63%. In [42], Hu *et al*. stated that chaetoglobosin A, the secondary metabolites produced by *Ch globosum* NK102 expressed the nematicidal activity *Meloidogyne incognita. Chaetomium globosum* NK102 also showed repellant second-stage juveniles. Chaetoglobosin A showed strongly adverse effects in secondary stage mortality of nematode with 99.8% at 300 μg /mL (LC_{50} = 77.0 μg/mL) at 72 h. Chaetoglobosin A and filtrates from *Ch. globosum* NK102 were not affect on egg hatching until 72 h. The filtrate treatments inhibited the penetration of second-stage juveniles at 12.5% dilution treatment. Chaetoglobosin A also inhibited the penetration of secondary stage and reduced the number of nematode eggs. Kooliyattil *et al.* [43] found *Ch. globosum* as a fungal parasite which isolated from egg of *Globodera pallida* (cyst nematode) in USA. It showed the greatest reduction of the infection by *G. pallida* in potato of about 76%. In [44], Khan *et al*. reported that endophytic *Ch. globosum* YSC5 showed nematicidal metabolite activities against the second stage juveniles of *Meloidogyne javanica*. Chaetoglobosin A, chaetoglobosin B and flavipin strongly inhibited (91.6, 83.8 and 87.4%, respectively) on mortality of the second stage juveniles at 200 μg/mL with LC_{50} values of 88.4, 107.7 and 99.2 μg/mL after 72 h, respectively, 3-methoxyepicoccone and 4,5,6-trihydroxy-7-methylphthalide moderately inhibited at 78.0 and 75.5%, respectively with LC_{50} values of 124.0 and 131.6 μg/mL, respectively. The promising metabolites Chaetoglobosin A, chaetoglobosin B significantly reduced nematode reproduction in pot experiment.

Chaetomium also showed insect control which was recorded by Zhou *et al.* [41] who stated that endophytic *Ch. globosum* showed negative affect on the fecundity of cotton aphids (*A. gossypii*) and beet armyworms (*Spodoptera exigua*). The beet army worms colovized on *Chaetomium* treated plants were with smally head capsule.

6. Bio-formulation of *Chaetomium* metabolites

Our research and development on *Chaetomium* biological products have been investigated since 1989. The first biological product of *Chaetomium* is contributed as a new broad spectrum biological fungicides from *Chaetomium* (Thailand Patent No. 6266, International Code: AO 1 N 25/12 and registered as Ketomium® biofungicide for plant disease control) which has been developed and improved from 22-strains of *Ch. cupreum* CC01-CC10 and *Ch. globosum* CG01-CG12 in the form of pellets, powder and liquid formulations. The practical integrated biological for plant pathogens is successfully introduced to farmers. Technology of disease control can be demonstrated either alone or integrated with other control measures. The products are scientifically proved not only for protection but also with curative effects as well as promoting plant growth. The formulations have been successfully used in infested field-soils integrated with cultural control for the long-term protection against durian and black pepper *(Piper nigram* L.) rot caused by *Phytophthora palmivora,* citrust rot caused by *Phytophthora parasitica* and strawberry rot caused by *Phytophthora cactorum,* tomato wilt caused by *Fusarium oxysporum* f. sp. *Iycopersici* and basal rot of corn caused by *Sclerotium rolfsii*. Further research is undergoing to develop bioactive metabolites from active strains of *Chaetomium* spp. for plant disease control and immunity.

7. Efficacy of *Chaetomium* to control brown leaf spot of rice

The active metabolites of *Chaetomium cochliodes* inhibited spore production of *Drechslera oryzae* causing brown leaf spot of rice var. Pittsanulok 2. *Chaetomium cochliodes* was reported to be a new antagonist to control brown leaf spot of rice var. Pittsanulok 2. Bio-fungicides produced from *Ch. cochliodes* were developed from active strain of *Ch. cochliodes* resulted in crude metabolites, powder, and liquid formulations which significantly inhibited the brown leaf spot of rice and increased plant growth. They significantly reduced leaf spot of rice var. Pitsanulok 2. The biopowder, crude metabolite and benlate-fungicide applied to rice seedlings at 40 days revealed plant height of 13, 13 and 12 cm, respectively when compared to the control (8 cm). The bio-powder, crude metabolite and benlate showed plant height of 50, 50 and 48 cm, respectively when compared to the control (21 cm) for 70 days. It was concluded that crude metabolite produced significantly higher rice growth parameters than the non-treated control [45].

8. Efficacy of *Chaetomium* to control fusarium wilt

The metabolites extracted from *Ch. cupreum* CC3003, *Ch. globosum* CG05, and *Ch. lucknowense* CL01 significantly inhibited growth and spore production of *F. oxysporum* NHP-Fusa-2 (**Figure 5**). The MeOH extract of *Ch. cupreum* CC3003 revealed to be more effective spore inhibition of *F. oxysporum* than the others which the ED_{50} was 85 μg/mL. The ED_{50} of hexane crude extract was 49 μg/mL, and EtOAc crude extract was 62 μg/mL in *Ch. globosum* CG05 and *Ch. lucknowense* CL01. Metabolites extracted from *Ch. cupreum* CC3003, *Ch. globosum* CG05, and *Ch. lucknowense* CL01 can be used to control tea wilt and root rot diseases caused by *Fusarium oxysporum.* It was the first time that *F. oxysporum* reported the causal pathogen of wilt and root-rot disease of tea in Vietnam [46].

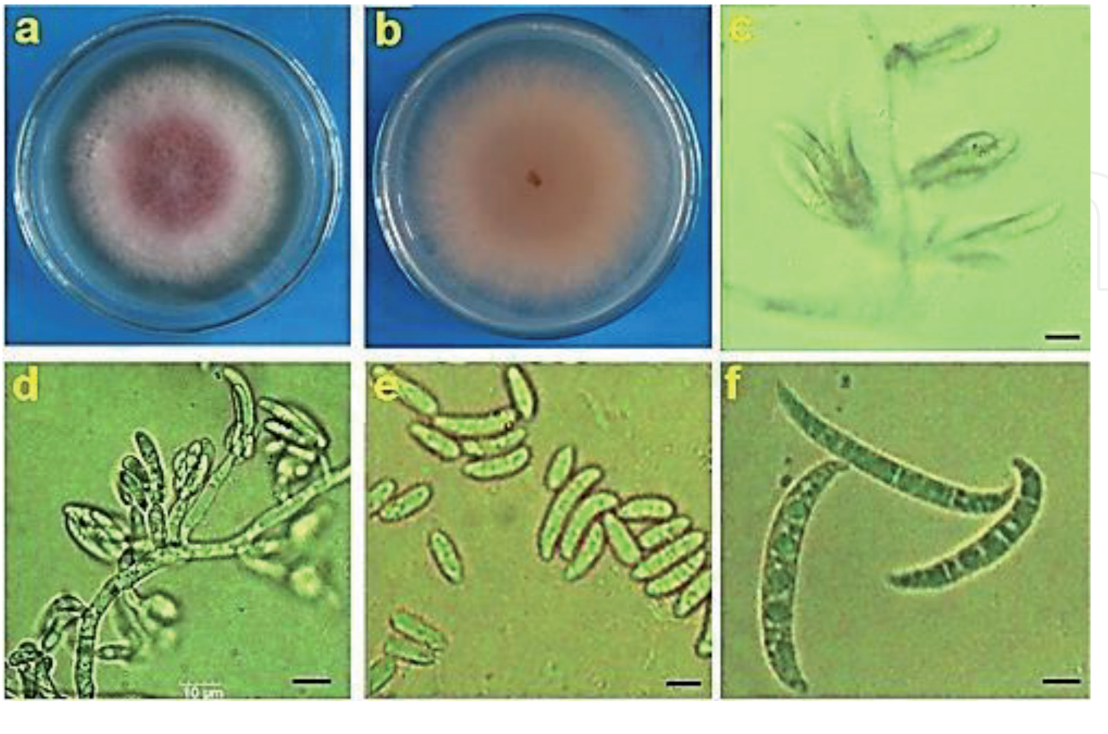

Figure 5.
Fusarium oxysporum *at 7-day-old culture on potato dextrose agar (colony) and water agar (conidia) (scale bar: 10 µm): (a) front surfaced colony; (b) back surfaced colony; (c) macroconidia on phialides; (d) microconidia on sporodochia; (e) microconidia; (f) macroconidia (source: [46]).*

The ED_{50} value of hexane crude extract from *Ch. globosum* N0802 inhibited *Fusarium oxysporum* f. sp. *lycopersici* NKSC01 causing tomato wilt var. Sida at 157 μg/mL. The crude hexane from *Ch. lucknowense* CLT and crude methanol from *Trichoderma harzianum* PC01 showed ED_{50} values of 188 and 192 μg/mL. The developed biofungicides - namely N0802, CLT and PC01 showed significantly higher wilt reduction of 44, 36 and 41%, respectively, than prochoraz fungicide (22%). All biofungicides significantly increased the yield more than prochoraz and inoculated control.

9. Efficacy of *Chaetomium* to control phytophthora root rot in citrus

Hung *et al.* [47, 48] found that pomelo rot caused by *Phythopthora nicotianae* in Thailand (**Figure 6**). Crude metabolites of *Ch. globosum*, *Ch. lucknowense*, *Ch. cupreum* showed antifungal activities against the growth of *P. nicotianae*, with ED_{50} of 3–101 μg/mL (**Figure 7**).

Chaetomium CG05, CL01, and CC3003 were reported to antagonize *P. nicotianae* showing inhibition between *Chaetomium* spp. and *P. nicotianae* before contact was made. The colonies of CG05 and CL01 made contact with *P. nicotianae* colonies without the clear zone of inhibition (**Figure 7**: A3 and B3). CG05, CL01, and CC3003 grew over colony of *P. nicotianae* in biculture plates in 30 days. Hyphae of *Chaetomium* penetrated or coiled around hyphae of *P. nicotianae*, maceration and discoloration of *P. nicotianae* KA1 colonies (from white to light yellowish-brown). Application of living *Chaetomium* spp. and metabolites reduced root rot of pomelo by 66–71% and increased plant weight by 72–85% compared to the non-treated control.

10. Biofungicide from *Chaetomium* elatum ChE01 against banana anthracnose caused by Colletotrichum musae

Colletotrichum musae is seriously caused banana anthracnose. *Chaetomium elatum* ChE01 showed antifungal activity to inhibit the growth of *C. musae* in

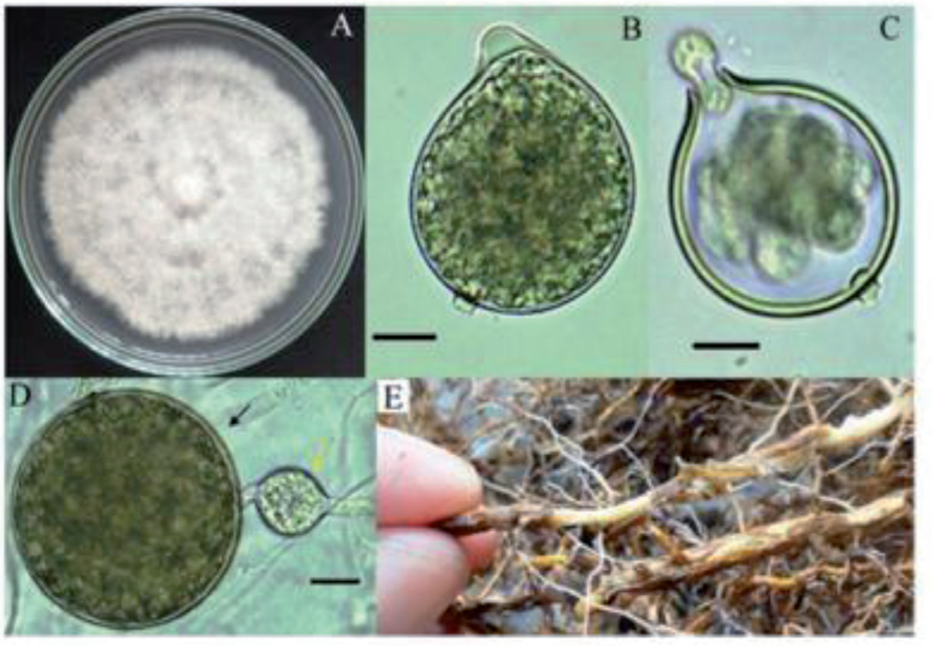

Figure 6.
Phytophthora nicotianae. *A, seven-day-old culture on PDA; B, C, sporangium with a short pedicel; D, Chlamydospore (black arrow) and hypha swelling (yellow arrow) (scale bars: B ~ D = 10 μm); E, root rot symptoms (source: [48, 47]).*

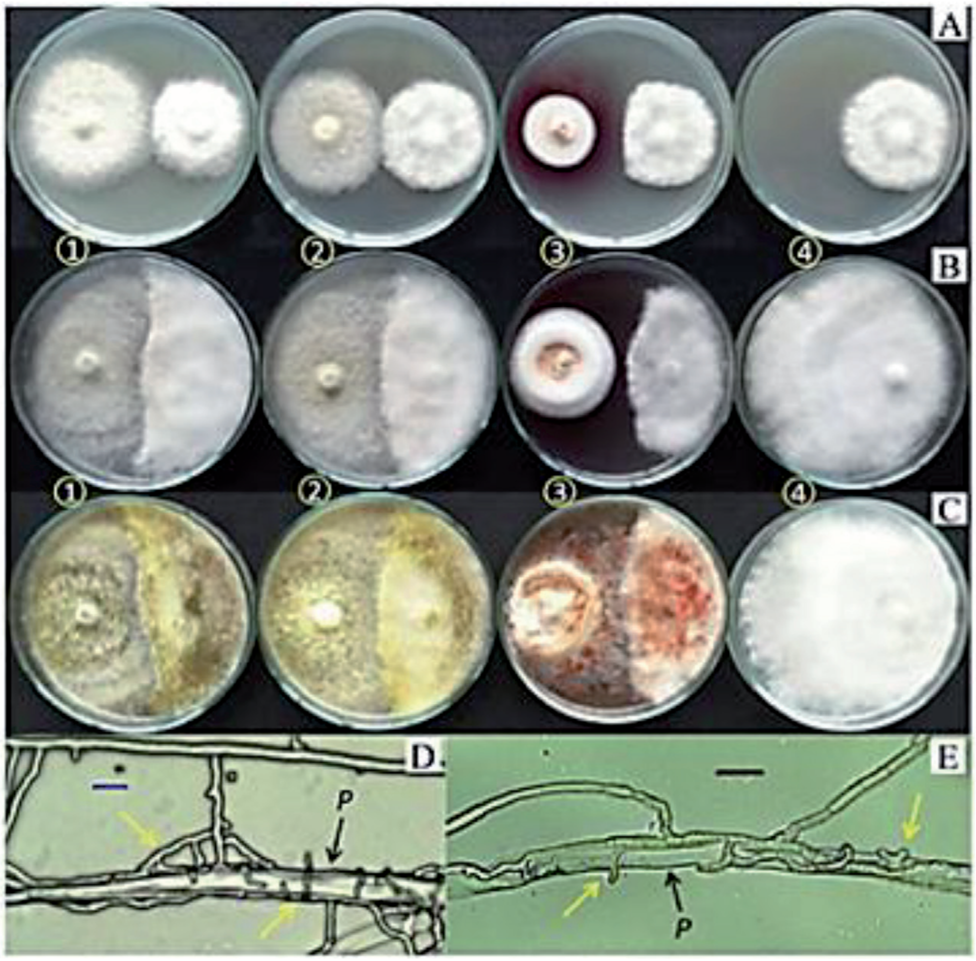

Figure 7.
Phytophthora nicotianae *in dual culture tests (A), 10 (B), and 30 days (C) incubation (1, 2, and 3:* P. nicotianae *[placed on the right side of the plates] against CG05, CL01, and CC3003, respectively; 4:* P. nicotianae *Alone); D, E,* Chaetomium *hyphae (yellow arrows) coiled around and grew inside hypha of* P. nicotianae *KA1 (P) (scale bars: D, E = 10 µm). Source: Hung et al. [48, 47].*

biculture evaluation over 60% and the spore production of *C. musae* was inhibited 57% in biculture trial. Metabolites from *Ch. elatum* ChE01 (crude methanol, crude ethyl acetate and crude hexane extracts) inhibited the spore production of *C. musae* with the ED_{50} values of 5, 7 and 19 μg/mL, respectively. The bio-fungicide produced from *Ch. elatum* ChE01 showed significantly better reduction of disease prevalence than benomyl fungicide. It is reported for the first time that biofungicide produced from *Ch. elatum* ChE01 controlled banana anthracnose caused by *C. musae* for the first time. *Chaetomium elatum* ChE01 produced a new chaetoglobosin V, two natural products, prochaetoglobosin III and prochaetoglobosin IIIed, six known chaetoglobosins B-D, F and G and isochaetoglobosin D [49] implying to a control mechnism of antibiosis. Moreover, crude methanol extract higher of *Ch. elatum* ChE01 showed significantly high spore inhibition of *C. musae* and the ED_{50} value was 5 μg/mL. The crude ethyl acetate and crude hexane extraction showed ED_{50} values 7 and 19 μg/mL, respectively. The higher concentration showed more inhibition of pathogens than the lower ones (**Figure 8**).

The coffee var. arabica plants were inoculated with 1 x 10^6 spores/mL suspension of the anthracnose pathogen (*Colletotrichum gloeosporioides*). Ten wounds were artificially made on leaves/ seedling with the fifth leaf from the top. Wounds were punctured with sterilized needles 10 times. The treatments were usually applied at 15 day intervals: T1 was inoculated with anthracnose pathogen, T2 was treated with a spore suspension of *Ch. cupreum* CC3003 at concentration of 1 x 10^6 spores/mL, T3 was treated with a biofungicide powder of *Ch. cupreum* CC3003 at

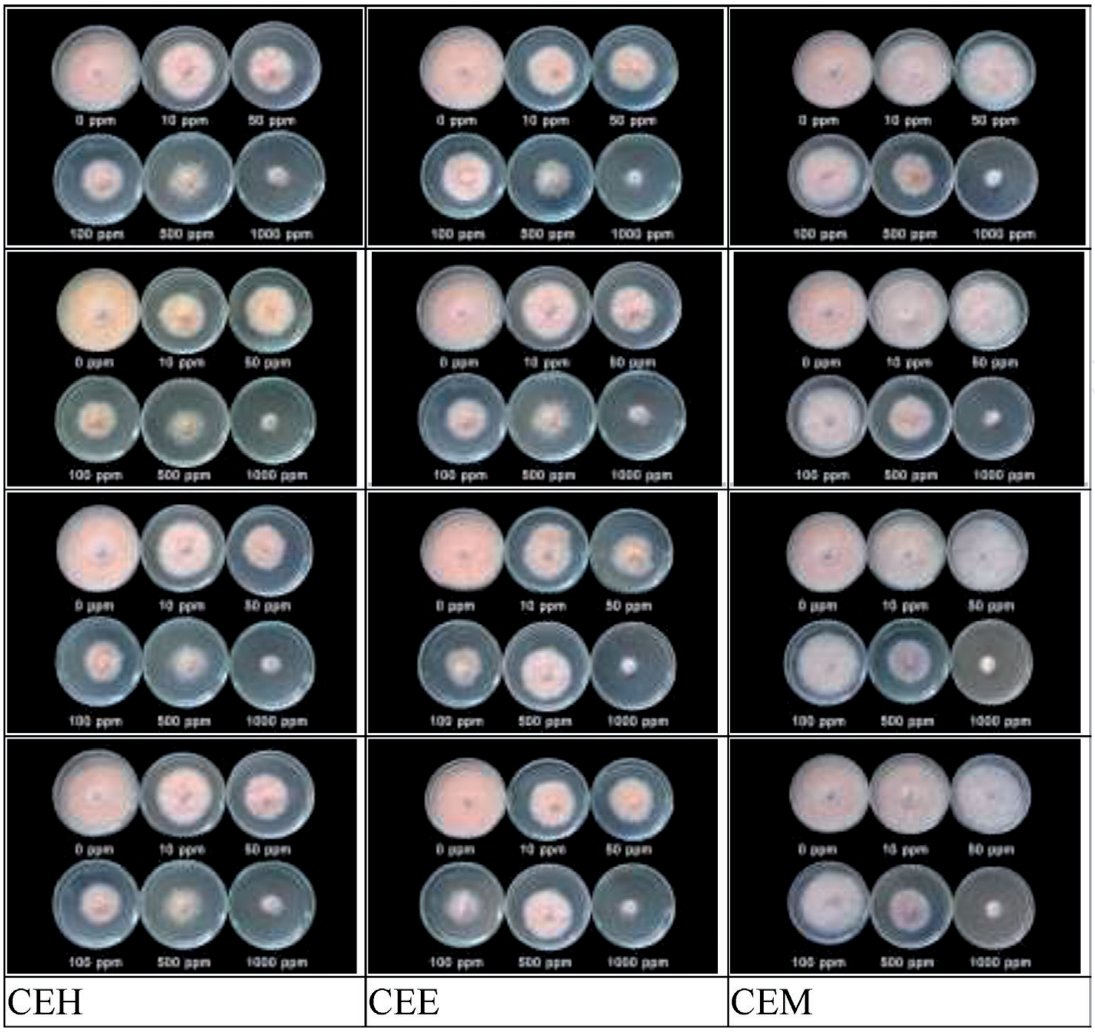

Figure 8.
Crude extracts of Chaetomium elatum *ChE01 testing to inhibit spore production of* Colletotrichum musae.

a concentration of 10 g/20 L of water, T4 was treated with nano- trichotoxin-A50 (made from *Trichoderma harzianum* PC01) and T5 was treated with nano-rotiorinol made from pure compound of *Ch. cupreum* CC3003). Nano- trichotoxin-A50 and nano-rotiorinol were produced by Dr. Kasem Soytong and Joselito Dar at the Biocontrol Research Laboratory, KMITL, Bangkok, Thailand [50]. *T. harzianum* PC01 reported to produce antibiotic polypeptides trichotoxin A50 [51]. Moreover, Vilavong and Soytong [52] reported that crude extracts with hexane, ethyl acetate and methanol from *Ch. cupreum* CC3003 expressed significant inhibition of *C. gloeosporioides* (coffee anthracnose) with ED_{50} values of 13, 11 and 28 μg/mL, respectively. Metabolites of *Ch. cupreum* CC3003 showed antifungal activity against *C. gloeosporioides* with abnormal appearance of spores of tested pathogen. A powder of the biofungicide produced from *Ch. cupreum* reduced anthracnose 54%.

11. Biodegradable nano-particles constructed from active natural products of different species of *Chaetomium* for immunity

Chaetomium species have been reported to degrade cellulolytic plant debris into soil to increase soil fertility as well as organic matter in the soil, and a specific isolate of *Ch. globosum* was reported to inhibit *Pyricularia oryzae* causing rice blast [4]. Soytong *et al.* [20] showed that chaetoglobosin C from *Ch. globosum* KMITL-N0805 actively inhibited several plant pathogens, such as *C. lunata* (leaf spot of corn), *Colletotrichum* sp. (citrus anthracnose), and *Fusarium oxysporum* f.sp. *lycopersici* (tomato wilt).

Kanokmedhakul *et al.* [25] reported that *Ch. globosum* KM ITL-N0802 produces a novel anthraquinone-chromanone compound, named chaetomanone, and known compounds as chaetoglobosin C and echinulin. Chaetomanone and echinulin were reported to be inhibitors of *M. tuberculosis* causing Tuberculosis of human being.

The biodegradable nanoparticles from natural products of active metabolites of *Chaetomium* spp. are further investigated and discovered as a new scientific investigation, namely microbial degradable nano-elicitors for inducing immunity in plants (the authors).

In recent years, scientists have interested nanoparticles possessing biological properties [53, 54]. The nanotechnology for agriculure is interested in various areas [55]. Plant disease control is to decrease or eliminate the nontarget effects either abiotic or biotic factors. Nano-sciences have become a new method to restructure materials at atomic level. Molecular nanotechnology can construct the organic materials into defined structures and atom by atom [56]. Natural products from *Chaetomium* species proved to be functioned for antifungal strategy against several plant pathogens [20]. The alternative disease control is to acheive, safe, effective, and environmentally friendly. The construction and characterization of copolymer nanoparticles loaded with bioactive compounds from *Chaetomium* species have been searched rather than toxic chemical pesticides. The natural products from *Chaetomium* spp. constructed to be fine particles at molecular level as degradable nanoparticles used to control plant disease and induce plant immunity. Biocontrol research unit, Faculty of Agricultural Technology, King Mongkut's Institute of Technology (KMITL), Bangkok, Thailand developed the research on biocontrol technology for years. Degradable nano-CGH, nano-CGE, and nano-CGM constructed from *Ch. globosum* KMITL-N0805 actively inhibited *Curvularia lunata* causing leaf spot disease of rice var. Sen Pidoa in Camobodia. The effective dose of 50% (ED_{50}) of degradable nano-CGH, nano-CGE, and nano-CGM were 1.21, 1.19, and 1.93 μg/mL, respectively at very low concentration was sufficeint to inhibit leaf spot pathogen of rice. These biodegradable nanoparticles actively penetrated to the pathogen cells causing to their disruption and distortion. Those pathogen inocula lost pathogenicity according to preliminary Koch's postulate test. The nano-CGH, nano-CGE, and nano-CGM inhibited spore production by 93%, 93%, and 84%, respectively and resulted antifungal activity against *C. lunata* with ED_{50} values of 1.2, 1.2, and 1.9 μg/mL, respectively.

The applications of degradable nano-CGH, nano-CGE, and nano-CGM to inoculated *C. lunata* on rice seedlings var. Sen Pidoa reduced disease incidence in pot experiments. Degradable nano-CGH and nano-CGM gave higher disease reduction of rice leaf spot caused by *C. lunata* (61%) than nano-CGE (54%). These nanoparticles significantly increased the height and number of tillers of the rice plants 60 days after treatment [57].

Ditta [58] stated that application of chemical pesticides causing climate change, and affected to urbanization, and natural resources. Those chemicals runoff or accumulate toxic pesticides in soil and it is needed to solve these problems immediately. However, there are still a few reports for the use of nanocarrier systems in agriculture [59]. Rai and Ingle [60] suggested that nanotechnology provides efficient to control diseases and insect pests. Ditta [58] added that nanotechnology has a great potential in agriculture due to its enhancing life quality, especially in crop production. Nanotechnology should be carefully evaluated for postive and negative impacts as done with any new technology. Some nanoparticles are formulated to contain chemical pesticides in colloidal suspensions or as powders, at the nano or micro scale that must be noticed. Soutter [56] stated that application of nanotechnology in agriculture should be done in precision farms and become the new "industrial revolution" in agriculture. Ditta [58] suggested that there is a

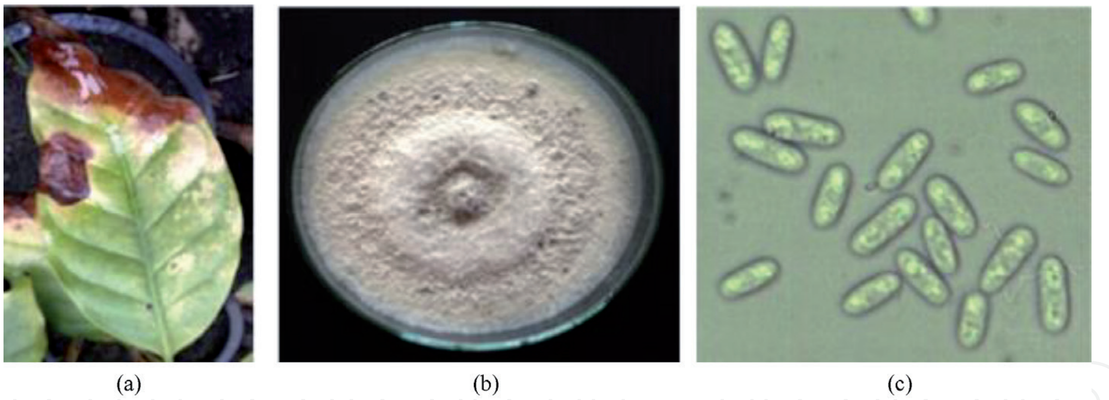

Figure 9.
Leaf anthracnose of coffee var. Arabica caused by Colletotrichum gloeosporioides *(A); pure culture on PDA at 20 days (B); and conidia, 400 X (C) source: Vilavong and Soytong [52].*

great potential for nanoscience and nanotechnology to face various challenges in agriculture. Perlatti *et al*. [61] expressed that nanoparticles serve as "magic bullets" that contains the bioactive substances from antagonistic fungi enable penetrating through cuticles and tissues, and constantly releasing active substances. The most popular shapes of nanomaterials being used for biocides delivery are nanospheres, nanocapsules, and nanogels. These preparations are advantageous in increasing the stability of active organic compounds, systemic activity, synergism, and specificity, with reducing foliar settling and leaching. The amount of pesticide dosage, number of applications, human exposure to pesticides, and environmental impacts are reduced. The nano formulation is employed in synthetic insecticides and alternative products such as herbal extracts and microorganisms to control insects.

Dar and Soytong [62] first characterized their electrospun materials through visual observation by the naked eye. It was observed that the control has white color. The size of the control nanoparticles ranged from 185 to 218 nm, while the extract-loaded nanoparticles was 241 nm. Tann and Soytong [63] reported that bioformulations and nano product from *Ch. cupreum* CC3003 showed a good control leaf spot of rice var. Sen Pidoa caused by *C. lunata*. Vilavong and Soytong [52] reported that the application of nano-rotiorinol, nano-trichotoxin and a spore suspension of *Ch. cupreum* reduced anthracnose incidence of 46, 43 and 18%, respectively while the inoculated control had high anthracnose disease caused by *Colletotrichum gloeosporioides* (**Figure 9**). The application of bio- formulation of *Ch. cupreum* in a powder form, nano-rotiorinol, and nano-trichotoxin to reduce coffee anthracnose was reported for the first time in Laos.

12. Testing bio-formulations and nano-products against anthracnose of coffee

The powder biofungicide of *Ch. cupreum* CC3003 at 0.5 g/L of water showed effectively control anthracnose of coffee which caused by *C. gloeosporioides,* resulted a disease index (DI) of 0.9 after application. The biodegradable nano-rotiorinol, nano- trichotoxin and living spores of *Ch. cupreum* gave DI of 1.07, 1.14 and 1.62, respectively compared to non-treated control (1.99). However, a biofungicide powder produced by *Ch. cupreum* CC3003 showed the highest disease reduction of 54%. The biodegradable nano-rotiorinol resulted in decrease of anthracnose diseasae 46%. The biodegradable nano-trichotoxin and spores of *Ch. cupreum* decreased the anthracnose disease of 42 and 18%, respectively, when compared to the non-treated control. Biofungicide produced from *Ch. cupreum* CC3003 provided a good control effectively to coffee anthracnose caused by *C. gloeosporioides*. Moreover, it is concluded that biodegradable nano-microbial elicitors from *Chaetomium* and *Trichoderma* PC01 was expressed to induce plant immunity through phytoalexin production against anthracnose of coffee caused by *C. gloeosporioides* [52].

13. Natural product of nano-particles constructed from *Chaetomium* spp. for rice blast disease control

Song *et al*. [64] stated that agricultural nanotechnology is started to be a new tool for plant disease management by restructuring natural active metabolites at the molecular level. Metabolites' nanoparticles contain bioactive compounds from natural products that can rapidly and effectively penetrate through plant cells and can increase the stability of active compounds to control plant diseases. This research finding is a new scientific discovery suggesting that natural products of nano-particles derived from *Ch. cochliodes* isolate CTh05 actively controlled *Magnaporthe oryzae* isolate PO1causing rice blast for the first time.

Chaetomium cochliodes isolate CTh05 expressed actively against the rice blast pathogen, *Magnaporthe oryzae* isolate PO1 on rice var. RD57 in Thailand. The rice blast reduced infection (59%) after applying nano-CCoM at 7 *μ*g/mL, followed by nanoCCoE and nanoCCoH which reduced the blast infection of 57 and 50% respectively. But tricyclazole fungicide reduced blast infection of 55% in 30 days. Later, Song *et al.* [65] further reported on antifungal efficacy of microbial nano-particles constructed from *Ch. elatum*, *Ch. lucknowense* and *Ch. brasiliense* against rice blast pathogen in rice var. PSL 2 in Thailand. Nano particles of *Ch. elatum* (nano-CEE, nano-CEM and nano-CEH) inhibited sporulation of *M. oryzae* which the ED_{50} values of 7, 8 and 16 *μ*g/mL. respectively. The nano-CBH, nano-CBE and nano-CBM constructed from *Ch. brasiliense* suppressed sporulation of the blast pathogen with the ED_{50} values of 6, 9 and 13 *μ*g/mL, respectively. Nano-particles from *Ch. lucknowense* (nano-CLM, nano-CLE and nano-CLH) inhibited sporulation of rice blast pathogen with the ED_{50} values of 5, 7 and 10 *μ*g/mL, respectively. Interestingly, all tested nano-particles deriverd from *Chaetomium* caused pathogenicity lost of rice blast pathogen due to broken down of pathogen cells. The treated rice leaves with nano-CBH from *Ch. brasiliense* showed the Rf values of 0.05 and 0.28 which defined to produce Sakuranertin and Oryzalexin B as phytoalexin against blast disease. Our reserch findings have developed to be a natural product of nanoelicitor for rice blast immunity.

14. Concluding remarks

The scientific investigations have proved that *Chaetomium* biofungicide can be directly applied into rhizosphere soil. *Chaetoimum* biofungicide can be sucessfully applied to control of various diseases, except for downy mildew, powdery mildew and rust as they are obligate parasites. Hence, it can be applied to control plant diseases for good agricultural practices (GAP), pesticide free production (PFP), non agrochemical production (NAP) and organic agriculture (OA). *Chaetoimum* biofungicide and biostimulants are applied to implement integrated plant disease control. It is proved to be a biological agent for plant diseases which has been successfully integrated with other control measures for suitable disease control. *Chaetomium* biofungicide showed protective and curative effects in controlling plant disease and promoting plant growth. It has been successfully applied to the infested soils for the long-term protection against many plant diseases. The new bioactive compounds from *Ch. globosum*, *Ch. cupreum*, *Ch. elatum*, *Ch. cochliodes*, *Ch. brasiliense*, *Ch. lucknowense*, *Ch. longirostre,* and *Ch. siamense* are reported to inhibit plant pathogens. These active natural products from different strains of *Chaetomium* are further developed to be biodegradable nanoparticles from active metabolites as a new discovery of scientific investigation which used to induce plant immunity, namely microbial degradable nano-elicitors for inducing immunity in plants. The biodegradable nano-elicitors are developed to induce plant immunity through phytoalexin production in plants e.g. inducing tomato to produce alpha-tomaline against *Fusarium* wilt of tomato, capsidiol against chili anthracnose, sakuranitin against rice blast, scopletin and anthrocyaidin against *Phytophthora* or *Pythium* rot Durian and scoparone against *Phytophthora* or *Pythium* rot of citrus etc.

Chaetomium is a unique board spectrum biofungicide which is registered in Thailand, China, Laos, Vietnam, Cambodia, and BioAgriCert, IFOAM (International Federation of Organic Agriculture Movements). These countries contributed and tested *Chaetomium* biofungicide. At present, it is registered in Cambodia, Laos, Vietnam and being registered in China and BioAgriCert, IFOAM. Biodegardable nano-elicitors constructed from active metabolites from *Chaetomium* species are the new unique science for plant immunity which have been contributed in Thailand, Finland, Indonesia, India, Laos, Cambodia, Myanmar, Vietnam and China. Our experienced research investigation in this area of specialization would be contributed for sustainable development goals (SDGs) in agriculture.

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