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New discovery of natural product nanoparticles constructed from active metabolites from *Chaetomium brasiliense* for immunity to brown spot of rice var. RD 47

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Abstract Natural product nanoparticles constructed from active metabolites from *Chaetomium brasiliense* is firstly presented as a new elicitor for immunity to brown spot of rice var. RD 47 caused by *Drechslera oryzae.* The nano-CBH, nano-CBE, nano-CBM from *C. brasiliense* at 15 µg/ml are treated to rice leaves after 3 days expressed R_f of 0.61 which supposing to be Oryzalexin C. Moreover, ethyl acetate, hexane and methanol crude extract derived from *C. brasiliense* that shown to be inhibited *D. oryzae* which the ED_{50} were 0.24, 0.32 and 0.35 µg/ml, respectively. Nano-CBH, nano-CBE, nano-CBM inhibited *D. oryzae* with ED_{50} values of 5.86, 4.92, and 2.86 µg/ml, respectively. All nanoparticles derived from *C. brasiliense* were more actively responded to suppress the pathogen inoculum of *D. oryzae* at the lower concentration than crude extracts at lower concentrations.

Keywords: Phytoalexin, Brown leaf spot of rice, Natural product nanoparticles

Introduction

Rice (*Oryza sativa* L.) is widely consumed as staple food of human population (FAOSTAT, 2020). Brown leaf spot is caused by *Drechslera oryzae* which reported as one of the important pathogen of rice leading to yield loss. It causes blight symptoms starting in seedlings which reported to destroy the plants in between 10-58% (Rice Department, 2018; Savary *et al.,* 2000). The disease is controlled by chemical fungicides for years then pathogen becomes resistance to fungicides and causing unbalance agroecosystem as well as hazardous to humans and animals. Biological fungicides have been recorded to apply for plant disease control. *Chaetomium brasilense* CB01 is a fungus which belongs to Ascomycota which reported to be to control tomato wilt caused by

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Fusarium oxysporum f.sp. *lycopersici*. (Sibounnavong *et al.*, 2012). Phytoalexins are metabolites which produce by plant for immunity system (Ahuja *et al*., 2012; Gro ßkinsky *et al*., 2012). Phytoalexins are recorded into two major groups in rice, the first group includes oryzalexin A–F (Akatsuka *et al.*, 1983; Akatsuka *et al.*, 1985; Kato *et al.*, 1993; Kato *et al.* 1994; Kono *et al.*, 1984. and Sekido *et al*., 1986), oryzalexin S (Kodama *et al*., 1992a) and the second group includes sakuranetin (Kodama *et al*., 1992b). The objective was to investigated a new natural product metabolites from *Ch. brasilense* CB01 to induce phytoalexin against brown spot of rice var. RD 47 caused by *D. oryzae.*

Materials and methods

Antagonistic fungus

Chaetomium brasiliense CB01 were transferred to sterilzed potato dextrose agar (PDA) medium and incubation was done at room temperature, then periodically observed under compound microscope.

Pathogen

The symptoms of rice brown leaf spot var RD 47 was isolated using tissue transplanting method on water agar (WA), then a single colony was transferred to PDA until appear pure culture and observed in compound microscope.

Pathogenicity test

Pathogenecity experiment was performed by Completely Randomized Design (CRD) which repeated four times. The isolate of pathogen was used the detached leaf method following Koch's Postulate method for pathogenicity test. The biomass of pathogen was removed into sterilized distilled water to get spores which adjusted to 5×10^6 spores/ml by using haemacytometer. Rice seedlings var. RD 47 were grown in pots for 15 days. The wounded leaves were gentle pressed by carborandam (3 leaves/seedling) and were inoculated the spore suspension of pathogen. The inoculation on wounded leaves were put in moist chamber using plastic sheet and periodically observed disease incidence. The treated leaves with sterilize distilled water served as controls. Disease Index (DI) was rated according to International Rice Research Institute (IRRI, 2002) as follows:1 = no incidence, 2 = symptom on leaves, less than 1%, 3 = symptom on leaves, 1-3%, 4 = symptom on leaves, 4-5%, 5 = symptom on

leaves, 11-15%, 6 = symptom on leaves, 16-25%, 7 = symptom on leaves, 26-50%, 8 = symptom on leaves, 51-75%, and 9 = symptom on leaves, 76-100%.

Extraction method

The sterilized potato dextrose broth (PDB) medium was used to culture *Chaetomium brasiliense* which incubated at room temperature for 30 days, then collected the biomass and air-dried for 24 hours. The biomass was blended in electrical blender and brought to dissolved in equal volume of hexane for 5 days at room temperature and whatman filter paper was used to get culture filtrate. The filtrate was evaporated in rotary vacuum evaporator to yield crude hexane. The remaining marc from biomass was extracted by soaking in ethyl acetate, and methanol respectively using the above procedure to get ethyl acetate and methanol crude extracts for further experiments.

Crude extract test

The experiment was performed using factorial in Completely Randomized Design (CRD). It was repeated for four times. Treatment combination was set up as factor A represented solvents (crude hexane, crude ethyl acetate and crude methanol). Factor B represented concentrationsc (0, 50, 100, 500 and 1000 μg/ml.). Each treatment was dissolved the crude extract in 2% of dimethyl sulfoxide incorporated with PDA, then autoclaved at 121C, 15lbs/inch^2 for 30 minutes. The peripheral colony of pathogen was cut by cork borer (3 mm dia.) and culture agar plug was moved to the middle of PDA plate (5.5 cm in dia.) incorporated with each crude extract. All treatments were incubated at room temperature (27-30^0 C) to observe pathogen grew full in control plate. Colony and number of spores were measured. Data were analyzed by analysis of variance (ANOV). DMRT at P=0.01 and 0.05 were differentiated for treatment combination. Effective dose (ED_{50}) was calculated by Probit analysis.

Nanoparticles test

The experimental design was followed crude extract testing. Factor A was nanofibers, nano-CBH, nano-CBE and nano-CBM. Factor B was concentrations of 0, 1, 3, 5, 7 and 10 μg/ml.

Testing nanoparticles to induce plant immunity

Natural product nanoparticles from *C. brasiliense* CB01 was constructed according to the method of Soytong, K (personal communication). The 15 days rice seedlings var. RD 47 were inoculated with spore suspension of *D. oryzae* at $1x10^5$ spores/ml. The non-inoculated control was sprayed with sterilize distilled water. The experiment was done in Randomized Complete Block Design (RCBD). It was repeated four times. Treatments were non-inoculated control, treated with nano-CBH and pathogen and treated with nano-CBE and pathogen and treated with nano-CBM and pathogen and treated with Propiconazole (chemical) and pathogen. Phytoalexin was preliminary determined using thin layer chromatography (TLC). Rice seedlings var. RD 47 of 20 days were treated by inoculating spore suspension of pathogen at $1x10^5$ spores/ml, and prayed with nanoparticle solution at a concentration of 15 ppm. Samples of leaves were gentle collected at 3, 6 and 9 days after treatment. Fresh leaf sample was get 1.5 g in each sample, and brought to clean in methanol before cut into small pieces. Each sample was blended before soaking in 10 mL methanol in waterbath at 50 ℃ for 10 minutes. The sample was passed through Whatman filter paper No. 4 to get filtrate. The filtrate was evaporated with Rotary Vacuum Evaporator to get a purified crude extract. The purified crude extract was added 3 mL methanol. Phytoalexin was detected by running on TLC in the combination of solvents, benzene and ethyl acetate at the ratio of 10:1. The TLC tank was filled 2 mL. Each purified crude extract was spotted on TLC plates. Phytoalexin standard was used to compare. TLC plate was examined under UV light at 365 nm. It was soaked in anisaldehyde solvent, dried and heated until spots appeared. R_f value was calculated to compare with standard. The distance spot travels/ distance mobile phase travel is calculated for R_f value.

Results

Antagonistic fungus

Chaetomium brasiliense CB01 belongs to Ascomycotina and Chaetomiaceae, it reveals brown to black fruiting body or perithecia, covered with grey hairs, dark olive-brown terminal and lateral hairs, cyclindrical asci (8 ascospores /ascus) with uniseriate, light olive-brown, and broadly ovate (Figure 1).

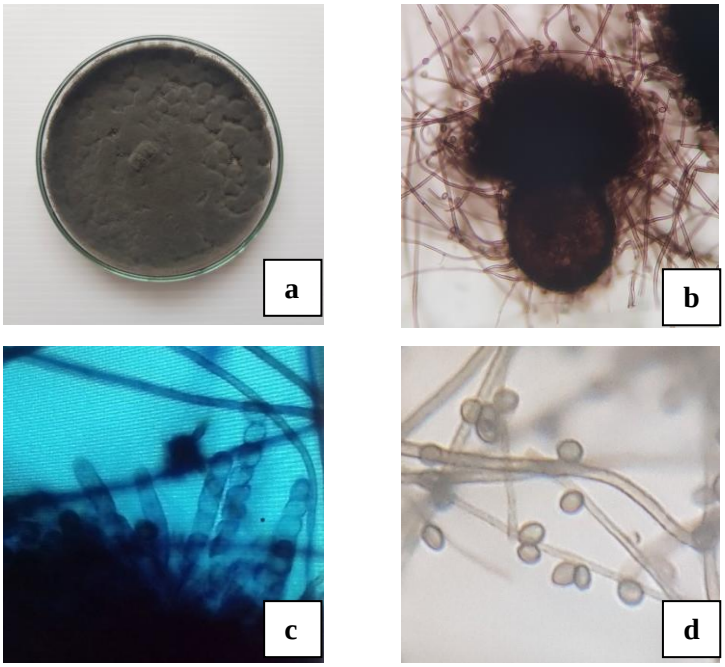


Figure 1. *Chaetomium brasiliense* a = colony, b = perithecium, c = asci, d = ascospores

Pathogen and pathogenicity test

Drechslera oryzae was isolated from brown spot of rice var RD 47. It belongs to Deuteromycotina, Dematiaceae. Pure culture is shown light brown in color when young, and dark brown in color when mature, septate hyphae and 5-7 septates/conidia (Figure 2). Pathogenicity was confirmed that symptoms appeared to bespots many light brown at the beginning and lesions are graduallly enlarged when mature leaf spots (Figure 3).

Figure 2. *Drechslera oryzae*, a = pure culture in PDA, b = conidiophore and conidium, c = conidium

Figure 3. Symptoms of brown leaf spots caused by *Drechslera oryzae* in rice var RD 47 after 7 days inoculation in pathogenicity test

Crude extract test

Crude ethyl acetate of *C. brasiliense* inhibited sporulation of *D. oryzae* which the ED_{50} of 0.24 µg/ml. Crude hexane and methanol extracts showed the ED_{50} of 0.32 and 0.35 µg/ml, respectively. Crude methanol, hexane and ethyl acetate at 1,000 ppm were non-significantly inhibited sporulation of pathogen at averaged of 99 %. Crude methanol, ethyl acetate and hexane extracts inhibited the mycelial growth of 83, 82, and, respectively 74 %.

Nanoparticles test

Nanoparticles of *C. brasiliense* were shown to be significantly suppressed sporulation of the tested pathogen at lower concentration than crude extract test. At the concentration 10 µg/ml of nano-CBH, nano-CBE, and nano-CBM inhibited sporulation of 85.25%, 77.86%, and 79.92%, respectively. Moreover, At concentrations of 1-10 µg/ml of nano-CBH, nano-CBE, and nano-CBM significantly suppressed the tested pathogen with ED_{50}values of 5.86, 4.92, and 2.86 µg/ml, respectively.

Testing nanoparticles to induce plant immunity

Nanopartcles of CBH, CBE and CBM from *C. brasiliense* treated to the inoculated rice seedings var var RD 47 which compared to the inoculated with pathogen (control) and Propiconazole are shown in Figure 4.

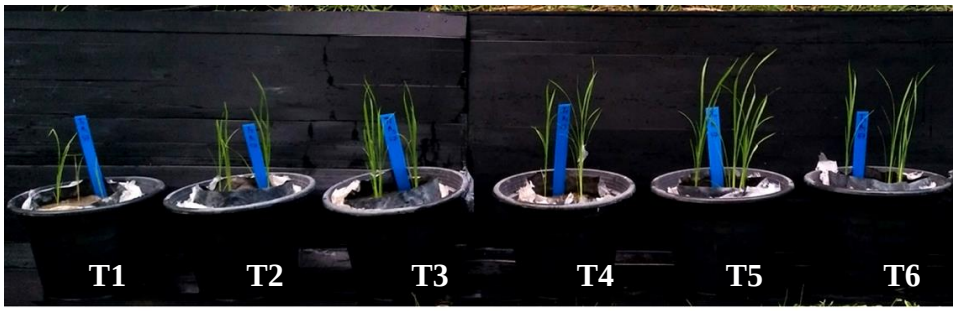


Figure 4. Testing nanaparticles derived from *Chaetomium brasiliense*

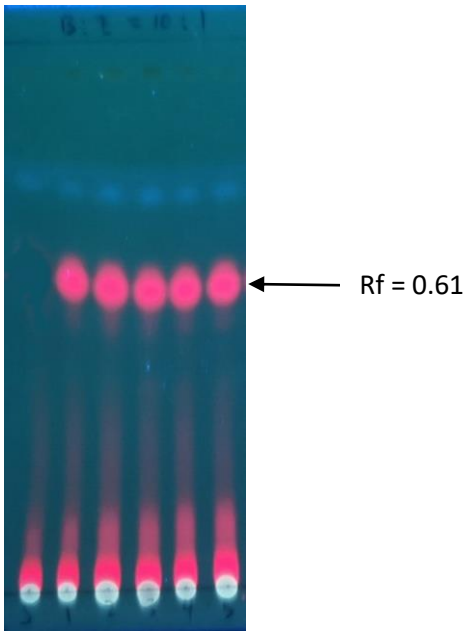


Figure 5. The presence of phytoalexin of Oryzalexin C at R_f value 0.61 on TLC plates when using mixture of benzene: ethyl acetate (10:1) under 365 nm UV light for leaves. The inoculated control with pathogen (T1), treated with phytoalexin standard (T2), treated with nano-CBH (T3), inoculated with pathogen and treated with nano-CBE(T4), inoculated with pathogen and treated with nano-CBM(T5) and inoculated with pathogen and treated with Propiconazole (T6)

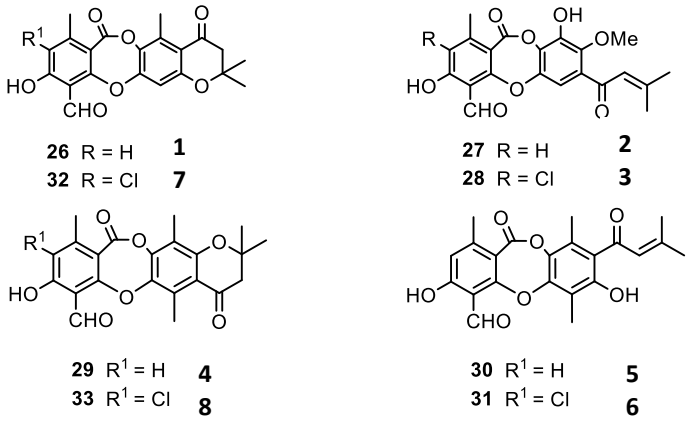
The inoculated control with pathogen (T1), treated with phytoalexin standard (T2), treated with nano-CBH (T3), inoculated with pathogen and treated with nano-CBE(T4), inoculated with pathogen and treated with nano-CBM(T5) and inoculated with pathogen and treated with Propiconazole (T6).

The rice leaves var var RD 47 treating with nano-CBH, nano-CBE, nano-CBM and Propiconazole were investigated for elucidating phytoalexin accumulation by thin layer chromatography (TLC). The treated with phytoalexin standard, nano-CBH, nano-CBE , nano-CBM from *C. brasiliense* and Propiconazole were found spots of treated crude extract of leaves on TLC plates in mixture of benzene: ethyl acetate at the ratio of 10:1 in 365 nm UV light which expressed spots on TLC plates, and showed the R_f of 0.61 which expecting to be Oryzalexin C (Figure 5).

Discussion

The brown leaf spot of rice (*D. oryzae*) was isolated from symptoms of rice var RD 47. It was identified as *Drechslera oryzae*, and proved to be pathogenic isolate as similar reports of Chaijuckam *et al.* (2019) and Marin-Felix *et al.* (2017). All crude extracts derived from *C. brasiliense* inhibited sporulation of the tested pathogen which the ED_{50} was 0.24 μg/ml, and crude hexane and crude methanol revealed the ED_{50} of 0.32 and 0.35 μg/ml, respectively. Tann and Soytong (2017) stated that crude extrtacts from *Chaetomium cupreum* resulted to control rice leaf spot in Cambodia. *Chaetomium brasiliense* CB01 used in this research finding is reported to produce mollicellins B (**1**), C (**2**), E (**3**), F (**4**) and mollicellins K-N (**5**-**8**) that compounds **1**-**3** and **5**-**7** inhibited *Plasmodium falciparum* and compound **30** inhibited *Candida albicans* and *Mycobacterium tuberculosis*. However, compounds **1**-**8** inhibited KB, BC1, NCI-H187 and cholangiocarcinoma cell lines (Khumkomkhet *et al.*, 2009). Song *et al.* (2020) reported that nano-particles derived from *Ch. brasiliense* inhibited rice blast pathogen in rice var. PSL 2. It is possible these bioactive substances act as control mechanism against brown leaf spot of rice.

Nanoparticles derived *C. brasiliense* were inhibited the inoculum *D. oryzae* at lower concentration than crude extracts. It is proved that nanofibers easy to enter into the pathogen cells and give fast response after treated into plants. Nano-CBH, nano-CBE, and nano-CBM at concentrations of 1-10 showed significantly inhibited *D. oryzae* at the ED_{50}values of 5.86, 4.92, and 2.86 μg/ml as similar report of Vilavong and Soytong (2017).



The research finding showed that rice leaves treated with nano-CBH, nano-CBE , nano-CBM from *C. brasiliense* and Propiconazole (chemical) expressed phytoalexin production using thin layer chromatography (TLC). The treated with phytoalexin standard, nano-CBH, nano-CBE , nano-CBM from *C. brasiliense* and Propiconazole (chemical) found spots of inoculated leaf extracts on TLC plates in the mixture of benzene : ethyl acetate at the ratio of 10:1) under 365 nm UV light resulting to be found spots on TLC plates with the R_f values of 0.61 as supposing to be Oryzalexin C. Withthis , Song *et al.* (2020) reported that nano-particles derived from *Ch. brasiliense* inhibited rice blast pathogen in rice. Those nano-CBH, nano-CBE and nano-CBM inhibited sporulation of the tested pathogen (*M. oryzae*) with the ED_{50} of 6, 9 and 13 μg/mL, respectively. The rice leaves were treated with nano-CBH expressed the Rf values of 0.05 and 0.28 which confirmed to be Sakuranertin and Oryzalexin C. The research finding was confirmed successful isolation of the oryzalexin C from treated the rice leaves with nanoparticles of CBH, CBE and CBM to the rice was achieved by Thin Layer Chromatography (TLC) which was examined by UV (Akatsuka *et al*.1985).

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Reference

Ahuja, I.; Kissen, R.and Bones, A. M. (2012). Phytoalexins in defense against pathogens. Trends Plant Sci. 17:73-90.

Akatsuka, T., Kodama, O., Kato, H., Sekido, H., Kono, Y. and Takeuchi, S. (1983). 3-hydrox-7-oxo-sandaracopimaradiene (oryzalexin A.), a new phytoalexin isolate from rice blast leaves. Agricultural and Biological Chemistry, 47:445-447.

Akatsuka, T., Kodama, O., Sekido, H., Kono, Y. and Takeuchi, S. (1985). Novel Phytoalexins (Oryzalexins A, B and C) Isolated from Rice Blast Leaves Infected with *Pyricularia oryzae.* Part I: Isolation, Characterization and Biological Activities of Oryzalexins. Agricultural and Biological Chemistry, 49:1689-1694.

Chaijuckam, P., Songkumarn, P. and Guerrero, J. J. G. (2019). Genetic Diversity and Aggressiveness of *Bipolaris oryzae* in North-Central Thailand. Applied Science and Engineering Progress, 12:116-125.

Gro ßkinsky, D. K.; van der Graaff, E.and Roitsch, T. (2012). Phytoalexin transgenics in crop protection—Fairy tale with a happy end? Plant Sci 195:54-70.

Kato, H., Kodama, O. and Akatsuka, T. (1993). Oryzalexin E, A diterpene phytoalexin from UV-irradiated rice leaves. Phytochemistry, 33:79-81.

Kato, H., Kodama, O. and Akatsuka, T. (1994). Oryzalexin F, a diterpene phytoalexin from UV-irradiated rice leaves. Phytochemistry, 36:229-301.

Khumkomkhet, P., Kanokmedhakul, S., Kanokmedhakul, K., Hahnvajanawong, C. and Soytong, K. (2009). Antimalarial and cytotoxic depsidones from the fungus *Chaetomium brasiliense.* Journal of Natural Products, 72:1487-91.

Kodama, O., Li, WX., Tamogami, S. and Akatsuka, T. (1992a). Oryzalexin S, a Novel Stemarane-type Diterpene Rice Phytoalexin. Bioscience, Biotechnology, and Biochemistry, 56:1002-1003.

Kodama, O.; Miyakawa, J.; Akatsuka, T.; Kiyosawa, S. (1992b). Sakuranetin, a flavanone phytoalexin from ultraviolet-irradiated rice leaves. Phytochemistry, 31:3807-3809.

Kono, Y., Takeuchi, S., Kodama, O. and Akatsuka, T. (1984). Absolute configuration of oryzalexin A and structures of its related phytoalexins isolated from rice blast leaves infected with *Pyricularia oryzae*. Agricultural and Biological Chemistry, 48:253-255.

Marin-Felix, Y., Senwanna, C., Cheewangkoon, R. and Crous, PW. (2017). New species and records of Bipolaris and Curvularia from Thailand. Mycosphere, 8:1556-1574.

Rice Department. (2018). Rice Knowledge Bank. Retrieved by Sep 5, 2018. http://www.ricethailand.go.th/rkb/disease%20and%20insect/index.php file=content.php&id=113.htm

Savary, S., Willocquet, L., Elazegui, F., Castilla, N. and Teng, P. (2000). Rice Pest Constraints in Tropical Asia: Quantification of Yield Losses Due to Rice Pests in a Range of Production Situations. Plant Disease, 84:357-369

Sekido, H., Endo, T., Suga, R., Kodama, O., Akatsuka, T., Kono, Y. and Takeuchi, S. (1986). Oryzalexin D (3, 7-Dihydroxy-(-+-)-sandaracopimaradiene), a New Phytoalexin Isolated from Blast-infected Rice Leaves. Journal of Pesticide Sciences, 11:369-372.

Sibounnavong, P., Sibounnavong, P.S., Kanokmedhakul S. and Soytong, K. (2012) Antifungal activities of *Chaetomium brasilense* CB01 and *Chaetomium cupreum* CC03 against F. oxysporum f. sp. lycopersici race 2. Journal of Agricultural Technology 8(3): 1029-1038.

Song, J. J., Soytong, K., Kanokmedhakul, S., Kanokmedhkul, K. and Poeaim, S. (2020). Natural Product of Nano-Particles Constructed from *Chaetomium* spp. to Control Rice Blast Disease Caused by *Magnaporthe oryzae*. International journal of agriculture & biology, 23:1013-1020.

Tann, H. and Soytong, K. (2017). Bioformulations and Nano Product from *Chaetomium Cupreum* CC3003 to Control Leaf Spot of Rice Var. Sen Pidoa in Cambodia. International Journal of Plant Biology, 7:6413.

UN Food and Agriculture Organization, Corporate Statistical Database (FAOSTAT). (2020). Retrieved by Oct 28, 2021. http://www.fao.org/faostat/en/#data/QC

Vilavong, S. and Soytong, K. (2017). Application of A New Bio-Formulation of *Chaetomium cupreum* For Biocontrol of *Colletotrichum gloeosporioides* Causing Coffee Anthracnose on Arabica Variety in Laos. AGRIVITA Journal of Agricultural Science, 39:303-310.

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