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# EFFECTS OF NANOPARTICLES LOADED WITH *Chaetomium globosum* KMITL-N0805 EXTRACTS AGAINST LEAF SPOT OF RICE var. Sen Pidoa

HUYLY TANN* and KASEM SOYTONG

*Department of Plant Production Technology, Faculty of Agricultural Technology,*
*King Mongkut's Institute of Technology Ladkrabang Bangkok,*
*Chalongkrung Road, Ladkrabang, Bangkok 10520, Thailand*
**Email: ajanhuylee@gmail.com*

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**ABSTRACT**

Nano-CGH, nano-CGE, and nano-CGM from *Chaetomium globosum* KMITL-N0805 expressed antifungal activity ($ED_{50}$ values of 1.21, 1.19, and 1.93ppm/mL, respectively) against *Curvularia lunata*, the causal agent of leaf spot disease of rice var. Sen Pidoa. It was demonstrated that these nanoparticles caused the disruption and distortion of the pathogen cells, leading to their loss of pathogenicity. Tests in a pot experiment showed that nano-CGH, nano-CGE, and nano-CGM could significantly control leaf spot of rice var. Sen Pidoa. Based on the disease severity index at 60 days after treatment, nano-CGH and nano-CGM resulted in higher disease reduction (61.54%) than nano-CGE (53.83%). From these findings, it can be concluded that nano-CGH, nano-CGE, and nano-CGM have the ability to decrease leaf spot disease of rice var. Sen Pidoa caused by *C.lunata.* Moreover, the research showed that all three types of nanoparticles significantly increased the height and number of tillers of the rice plant relative to the non-treated control.

**Key words**: Nanoparticles; brown leaf spot; *Chaetomium globosum*; rice var. Sen Pidoa

## INTRODUCTION

The search for effective alternative methods of plant disease control is mandated by the need to reduce or eliminate nontarget effects on both humans and the environment. Nanotechnology is a new methodology for building, restructuring, controlling, and devising materials at the molecular level. Molecular nanotechnology involves building organic materials into defined structures, atom by atom or molecule by molecule, often by self-assembly or self-organization (Soutter, 2012). Agricultural applications of nanotechnology have greatly advanced in recent years (Li *et al.*, 2011). Biologists are actively engaged in the synthesis and investigation of organic nanomaterials, including different kinds of nanoparticles possessing unusual optical, physical, and biological properties (Elibol *et al*., 2003; Salata, 2004). The possible uses of nanotechnology in agriculture are being explored. Precision farming, for example, along with nanodelivery systems is becoming the new "industrial revolution" in agriculture (Soutter, 2012). As such, there is great potential for nanoscience and nanotechnology in providing state-of-the-art solutions for various challenges faced by agriculture and society today (Ditta, 2012). Nanoparticles can serve as "magic bullets," containing bioactive substances from antagonistic fungi that can enable effective penetration through cuticles and tissues, allowing the slow and constant release of the active substances. The most popular shapes of nanomaterials being used for biocides delivery are nanospheres, nanocapsules, and nanogels (Perlatti *et al*., 2013). Nanotechnology can provide green and efficient alternatives for the management of diseases and insect pests in agriculture without compromising nature (Rai & Ingle, 2012). Consequently, nanotechnology has great potential in agriculture because its applications can enhance the quality of life, especially in food crop production systems. Nevertheless, although it may play a very important role in the development of any nation, nano-technology should be carefully evaluated as is done with any new technology.

* To whom correspondence should be addressed.

Some nanoparticles have been formulated to contain pesticides in colloidal suspensions or as powders, at the nano or micro scale (Ditta, 2012). These preparations have advantages, such as increasing the stability of active organic compounds, systemic activity, synergism, and specificity, and reducing foliar settling and leaching (Perlatti *et al*., 2013). As a consequence, the amount of pesticide dosage, number of applications, human exposure to pesticide, and environmental impact are reduced. Nanoformulation shave been employed not only for synthetic insecticides but also in alternative products such as natural products (herbal extracts) and microorganisms to control insects (Perlatti *et al*., 2013). Current issues like climate change, urbanization, sustainable use of natural resources, and runoff and accumulation of toxic pesticides, herbicides, and fertilizers need to be addressed immediately (Ditta, 2012). However, relatively few studies have reported the use of nanocarrier systems in agriculture (Nguyen *et al*., 2012). The use of bioactive compounds from different *Chaetomium* species has been proven to be an effective antifungal strategy against several plant pathogens (Soytong *et al*., 2001). In as much as the quest for safe, effective, and environmentally friendly methods of controlling plant pathogen is highly desired, the construction and characterization of copolymer nanoparticles loaded with bioactive compounds rather than toxic pesticides are needed. The objective of this research was to design nanoparticles from active compounds of *Chaetomium globosum* KMITL-N0805 and to test their effectiveness in controlling *Curvularia lunata*, the causative agent of leaf spot of rice var. Sen Pidoa.

## MATERIALS AND METHODS

### Preparation and characterization of the nanoparticles

The crude hexane (CGH), ethyl acetate (CGE), and methanol (CGM) extracts of *Ch. globosum* KMITL-N0805 were separately incorporated into polylactic acid (PLA)-based nanoparticles through electrospinning (Dar & Soytong, 2014). Each extract was first dissolved in two drops of dimethyl sulfoxide (DMSO) and heated to dissolve completely. Then, the extract was mixed with 2g of PLA nanoparticles that had been completely dissolved in 10 mL of tetrahydrofuran by heating. The resulting mixture was loaded into a syringe and placed into the electrospinning setup. The tip of the syringe was clipped to the positive pole while aluminum foil was clipped to the negative pole and served as the collector. The voltage used was 25 30 kV. Nanoparticles (i.e., PLA alone) containing no active compound (i.e., *Ch.globosum* extract) were also made and served as the controls. The product was carefully scraped from the aluminum foil and stored in tightly capped bottles. The characteristics of the nano-CGH, nano-CGE, and nano-CGM were noted by the naked eye and viewed under a scanning electron microscope, and the properties were analyzed by Fourier-transform infrared spectroscopy (FTIS).

### Testing the nanoparticles derived from *Chaetomium globosum* KMITL-N0805 extracts *in vitro*

Three types of nanoparticles were tested for their inhibition of the rice plant fungal pathogen *C.lunata*. The test was performed by using two-factorial experiments in a completely randomized design (CRD) with four replications. Factor A represented the nanoparticles; namely, the inoculated control, nano-CGH, nano-CGE, and nano-CGM. Factor B represented the concentrations of the nanoparticles; namely, 0, 1, 5, and 10ppm/mL. Each each type of nanoparticle was dissolved in 2% DMSO and mixed into potato dextrose agar (PDA) before autoclaving. The tested pathogen *C.lunata* was cultured on PDA and incubated at room temperature for 5 days. The colony margin was then cut using a 3-mm-diameter sterilized cork borer, and the agar plug of the pathogen was transferred to the middle of PDA plates containing each concentration of nanoparticles. The plates were incubated at room temperature (28–30°C) for 4 days. The number of *C. lunata* spores produced were counted with a hemacytometer under a microscope, and the percentage of inhibition was calculated. Data were statistically computed by analysis of variance (ANOVA). Treatment means were compared by Duncan's multiple range test (DMRT) at $P$=0.05 and $P$=0.01. The effective dose ($ED_{50}$) was computed by using probit analysis (Sibounnavong *et al*., 2012).

### Testing the nanoparticles against *Curvularia lunata in vivo*

This pot experiment was done by using a CRD with four replications. The test products were Non-treated control, nano-CGH, nano-CGE, and nano-CGM at the concentration of 10 ppm/mL. The leaves of 20-day-old seedlings of rice var. Sen Pidoa planted in pots were inoculated with $1 \times 10^6$ spore/mL of *C. lunata*. The disease severity index (DSI) on the leaves was scored as follows: 0 = no symptom; 1 = 1–15% infection; 2 = 26–50% infection; 3 = 51–75% infection; and 4 = 76–100% infection. Disease reduction (DR) was defined as follows:[(DSI in the Non-treated control – DSI in treatment) ÷ DSI in Non-treated control] × 100. Additional observations included those of the plant

height and number of tillers. The data were statistically computed by ANOVA, and treatment means were compared with DMRT at $P$= 0.05 and $P$=0.01. Additionally, the structures of treated and non-treated spores were compared under a compound microscope.

## RESULTS

### Characterization of the nanoparticles

The nanoparticles, comprising PLA loaded with crude extracts from *Ch. globosum* KMITL-N0805 and electrospun at 25–30 kV, were visually characterized. Nano-CGE was yellowish in color (Fig. 1). Scanning electron microscope images revealed that the nanoparticles measured 241 nm in diameter, which was verified by the FTIS analysis. On the other hand, the particle size of the control (PLA alone) ranged from 185 to 218 nm.

### Testing the nanoparticles derived from *Chaetomium globosum* KMITL-N0805 extracts *in vitro*

The results showed that nano-CGH, nano-CGE, and nano-CGM, eachat a concentration of 10ppm/mL, inhibited spore production by 92.70%, 93.44%, and 84.17%, respectively. These nanoparticles expressed antifungal activity against *C. lunata* with $ED_{50}$values of 1.21, 1.19, and 1.93 ppm/mL, respectively (Table 1). All three types of nanoparticles caused disruption and distortion of *C.lunata* spores, leading to loss of their pathogenicity (Fig. 2).

### Testing the nanoparticles against *Curvularia lunata in vivo*

The potential of these nanoparticles to control leaf spot of rice var. Sen Pidoa caused by *C. lunata* was investigated in a pot experiment. The results showed that nano-CGH, nano-CGE, and nano-CGM could significantly control leaf spot formation at 30, 45, and 60 days after treatment. The DSI at 30 days was the lowest with nano-CGM(DSI = 2.25), followed by nano-CGH(DSI = 2.75) and nano-CGE (DSI = 2.75). All treatments were significantly different with the non-treated control (DSI = 3.62) (Table 2). After treatment for 45 days, the effects of the nanoparticles were not significantly different from one another (DSI values of 2.50, 2.68, and 2.75, respectively), but were significantly different when compared with the non-treated control (DSI = 4.12). After treatment for 60 days, nano-CGH and nano-CGM were not significantly different in DSI value (DSI = 2.25), being lower than that of nano-CGE (DSI = 2.50), and all three nanoparticles still had much lower DSI values than the non-treated control (DSI = 4.25). After treatment for 30 days, nano-CGM had given the best DR effect (37.85%), which was significantly higher than the effects of nano-CGH and nano-CGE (24.03%). At 45 days after treatment, nano-CGH gave the best DR value (39.32%), where as the values for nano-CGE and nano-CGM were not significantly different from each other (34.95% and 33.25%, respectively). Moreover, at 60 days after treatment, nano-CGH and nano-CGM gave a higher DR (47.09%) than nano-CGE (41.18%). The increase in plant height after 30 days was highest with nano-CGH (1.79% increase) followed by nano-

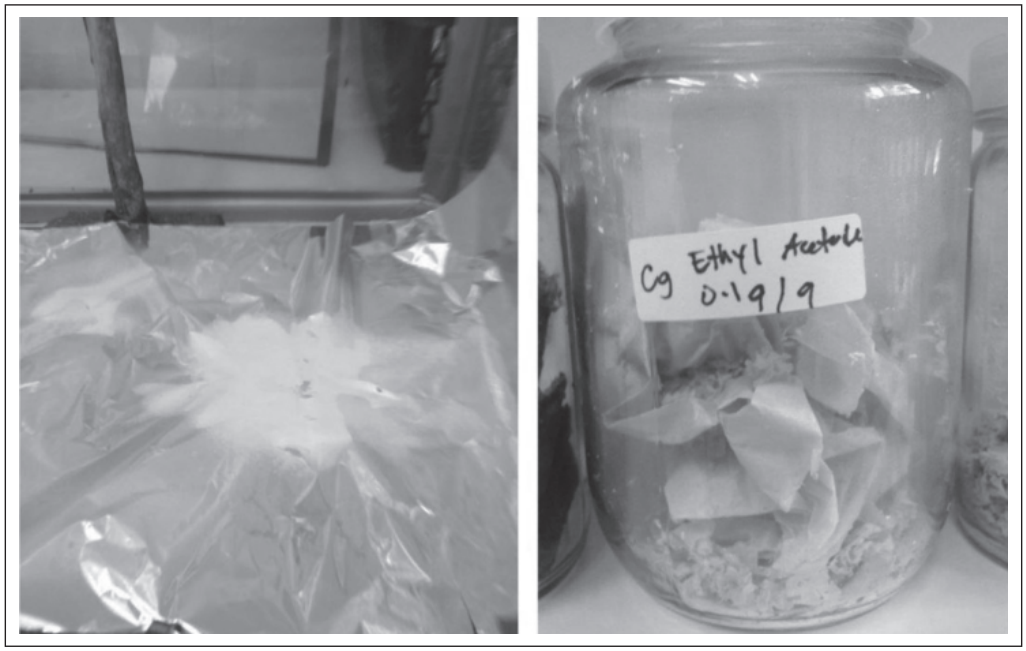


**Fig. 1**. The nanoparticles from *Chaetomium globosum* KMITL-N0805 extracts were yellowish in color.

**Table 1.** Effects of nano-CGH, nano-CGE, and nano-CGM on conidia formation by *Curvularia lunata*

| Type of nanoparticle | Concentration (ppm/mL) | Colony diameter (cm) | Number of conidia ($\times 10^6$) | Conidia inhibition (%) | $ED_{50}$ (ppm/mL) |
|---|---|---|---|---|---|
| Nano-CGH | 0 | 5.00 a | 6.85 a | – | |
| | 1 | 4.90 b | 4.25 d | 37.95 | |
| | 5 | 4.78 e | 1.80 f | 73.72 | |
| | 10 | 4.65 h | 0.50 g | 92.70 | 1.21 |
| Nano-CGE | 0 | 5.00 a | 6.10 b | – | |
| | 1 | 4.87 bc | 4.55 cd | 25.40 | |
| | 5 | 4.77 de | 2.50 ef | 59.01 | |
| | 10 | 4.69 f | 0.40 g | 93.44 | 1.19 |
| Nano-CGM | 0 | 5.00 a | 5.15 c | – | |
| | 1 | 4.88 bc | 2.89 e | 43.88 | |
| | 5 | 4.77 de | 2.30 ef | 55.33 | |
| | 10 | 4.69 f | 0.30 g | 84.17 | 1.93 |
| CV (%) | | 3.56 | 8.44 | – | |

Data are the average of four replications. Means followed by a common letter in each column are not significantly different by Duncan's multiple range test at $P = 0.01$. Nano-CGH = Nanoparticles which derived from crude hexane extracts of *C. globosum*, Nano-CGE = Nanoparticles which derived from crude Ethyl Acetate extracts of *C. globosum*, Nano-CGM = Nanoparticles which derived from crude Methanol extracts of *C. globosum*, CV = Coefficient of Variation.

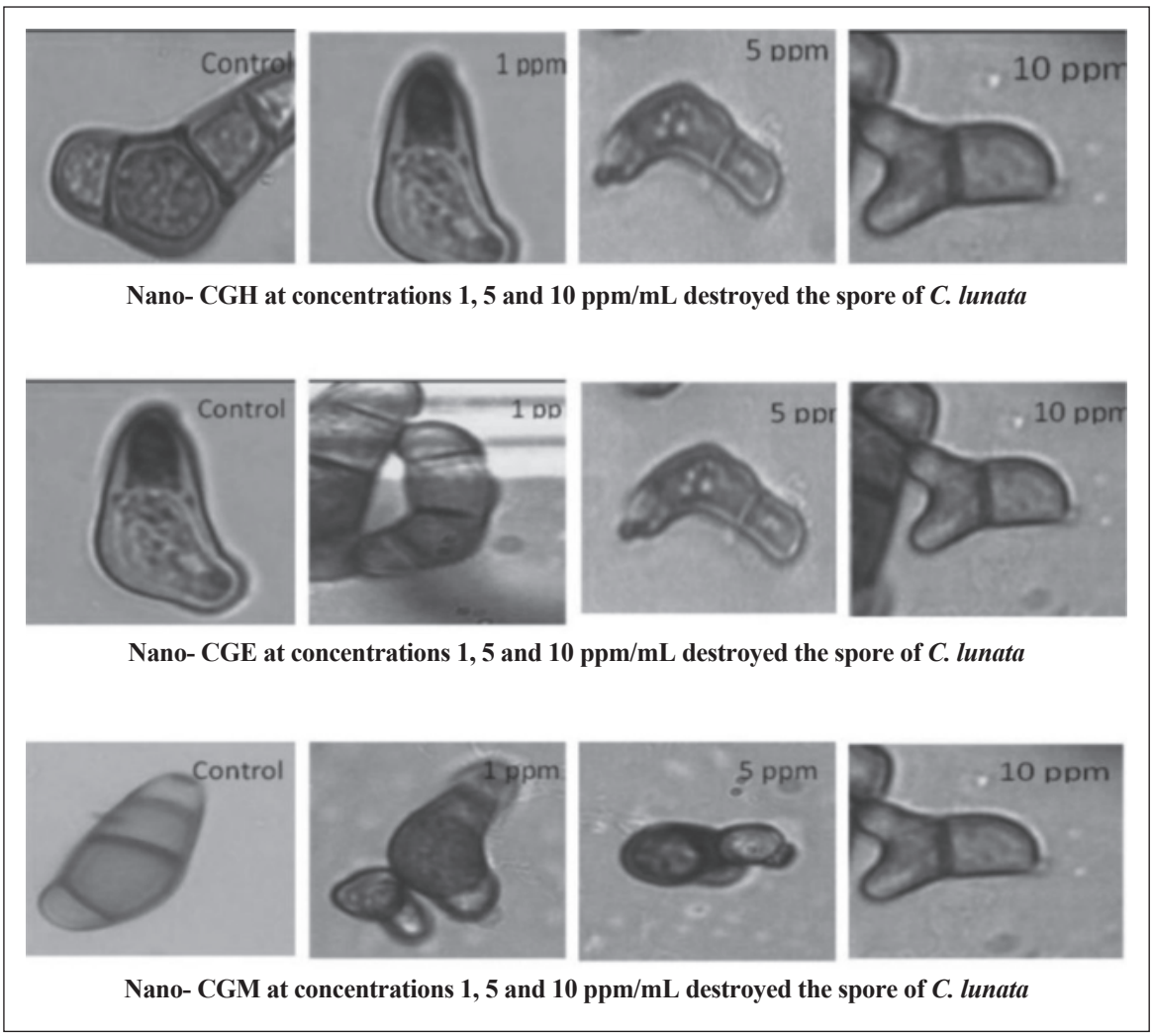


**Fig. 2**. Effects of the nanoparticles on spores of *Curvularialunata*, leading to loss of pathogenicity.

**Table 2.** Disease severity index and disease reduction after treatment of rice var. Sen Pidoa leaves with nanoparticles containing *Ch. globosum* KMITL-N0805 extracts

| Treatments | DSI | | | DR (%) | | |
|---|---|---|---|---|---|---|
| | 30 days | 45 days | 60 days | 30 days | 45 days | 60 days |
| Non-treated control | 3.62a | 4.12a | 4.25a | – | – | – |
| Nano-CGH | 2.75b | 2.5b | 2.25c | 24.03b | 39.32a | 47.06a |
| Nano-CGE | 2.75b | 2.68b | 2.50b | 24.03b | 34.95ab | 41.18b |
| Nano-CGM | 2.25c | 2.75b | 2.25c | 37.85a | 33.25b | 47.06a |
| CV(%) | 2.48 | 4.83 | 2.21 | 6.47 | 9.16 | 4.43 |

Data are the average of four replications. Means followed by a common letter in each column are not significantly different by Duncan's multiple range test at $P = 0.01$.
DSI = Disease Severity Index, DR = Disease Reduction, Nano-CGH = Nanoparticles which derived from crude hexane extracts of *C. globosum*, Nano-CGE = Nanoparticles which derived from crude Ethyl Acetate extracts of *C. globosum*, Nano-CGM = Nanoparticles which derived from crude Methanol extracts of *C. globosum*, CV = Coefficient of Variation.

**Table 3.** Effects of nanoparticles containing extracts of *Chaetomium globsum* KMITL-N0805 on plant height of rice var. Sen Pidoa after transplantation

| Treatments | Plant height (cm) | | | Increase in plant height (%) | | |
|---|---|---|---|---|---|---|
| | 30 days | 45 days | 60 days | 30 days | 45 days | 60 days |
| Non-treated control | 14.78a | 15.90c | 24.63b | – | – | – |
| Nano-CGH | 15.05a | 17.93ab | 25.34a | 1.79 a | 11.32b | 2.80a |
| Nano-CGE | 14.90a | 17.28bc | 25.09a | 0.80 c | 7.98c | 1.83b |
| Nano-CGM | 14.93a | 18.26a | 24.93ab | 1.00b | 12.92a | 1.20b |
| CV(%) | 1.12 | 3.60 | 0.71 | 5.49 | 8.61 | 19.69 |

Data are the average of four replications. Means followed by a common letter are not significantly different by Duncan's multiple range test at $P = 0.01$. Nano-CGH = Nanoparticles which derived from crude hexane extracts of *C. globosum*, Nano-CGE = Nanoparticles which derived from crude Ethyl Acetate extracts of *C. globosum*, Nano-CGM = Nanoparticles which derived from crude Methanol extracts of *C. globosum*, CV = Coefficient of Variation.

**Table 4.** Effects of nanoparticles containing *Chaetomium globosum* KMITL-N0805 extracts on tillers of rice var. Sen Pidoa after transplantation

| Treatments | Number of tillers | | | Increase of tillers (%) | | |
|---|---|---|---|---|---|---|
| | 30 days | 45 days | 60 days | 30 days | 45days | 60 days |
| Non-treated control | 1.50c | 4.68c | 12.31d | – | – | – |
| Nano-CGH | 2.18b | 7.06a | 15.87a | 31.19b | 33.71a | 22.43a |
| Nano-CGE | 2.68a | 5.25b | 14.31b | 44.02a | 10.85 b | 13.97b |
| Nano-CGM | 2.06b | 5.18b | 13.56c | 27.18b | 9.65 b | 9.21c |
| CV(%) | 14.59 | 3.54 | 1.83 | 5.51 | 6.56 | 5.04 |

Data are the average of four replications. Means followed by the same letter are not significantly different by Duncan's multiple range test at $P = 0.01$. Nano-CGH = Nanoparticles which derived from crude hexane extracts of *C. globosum*, Nano-CGE = Nanoparticles which derived from crude Ethyl Acetate extracts of *C. globosum*, Nano-CGM = Nanoparticles which derived from crude Methanol extracts of *C. globosum*, CV = Coefficient of Variation.

CGM (1%) and nano-CGE (0.80%). After 45 days, nano-CGM gave the highest increase in plant height (12.92%), followed by nano-CGH (11.32%) and nano-CGE (7.98%). After 60 days, nano-CGH had the best increase in plant height at 2.80%, compared with nano-CGE (1.83%) and nano-CGM (1.20%) (Table 3). The number of tillers after 30 days increased the most with nano-CGE (44.02%), followed by nano-CGH (31.19%) and nano-CGM (27.18%). After 45 days, nano-CGH gave the highest increase in the number of tillers (33.71%), followed by nano-CGE (10.85%) and nano-CGM (9.65%). After 60 days, nano-CGH gave the highest increase in number of tillers (22.43%), followed by nano-CGE (13.97%) and nano-CGM (9.21%)(Table 4).

## DISCUSSION

In this study, crude solvent extracts from *Ch. globosum* KMITL-N0805 were incorporated into PLA via electrospinning at 25–30 kV to obtain bioactive compound-loaded nanoparticles for characterization. Similar to Dar & Soytongn (2014), who first characterized their electrospun materials through visual observation by the naked eye, it was observed that the control has a white color. The size of the control PLA nanoparticles ranged from 185 to 218 nm, where as that of the extract-loaded nanoparticles was 241 nm. Results showed that nano-CGH, nano-CGE, and nano-CGM expressed antifungal activity against *C. lunata* at $ED_{50}$ values. Kanokmedhakul *et al* (2002) reported that *Ch. globosum* KM ITL-N0802 produces a novel anthraquinone-chromanone compound, named chaetomanone, as well as known compounds such as chaetoglobosin C and echinulin. They found that chaetomanone and echinulin were active to wards *Mycobacterium tuberculosis*. Soytong *et al* (2001) stated that chaetoglobosin C from *C. globosum* KMITL-N0805 can actively inhibit several plant pathogens, such as *C. lunata* (leaf spot of corn), *Colletotrichum* sp. (citrus anthracnose), and *Fusarium oxysporum* f.sp. *lycopersici* (tomato wilt). Moreover, there are reports that the enzyme CHI46 produced by *C. globosum* can efficiently degrade the cell walls of the phytopathogenic fungi *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Sclerotinia tritici*, and *Phytophthora sojae*, indicating that this enzyme may be involved in the biocontrol mechanism of *C. globosum* (Liu *et al*., 2008). The pot experiments of this present study revealed that nano-CGH, nano-CGE, and nano-CGM from *C. globosum* KMITL-N0805 significantly reduced leaf spot disease of rice var. Sen Pidoa at 30, 45, and 60 days after treatment. Previous reports on *C. globosum* as an important biocontrol fungus have indicated that it can inhibit spot blotch of wheat (Aggarwal *et al.*, 2004), suppress the development of rice blast and wheat leaf rust (Park *et al*., 2005), and reduce the primary inoculum of *Diaporthe phaseolorum* f.sp. *meridionalis* in soil-surface soybean stubble under field conditions (Dhingra & Sinclair, 1987). Moreover, *Chaetomium* spp. have been reported to degrade cellulolytic plant debris to increase organic matter in the soil, and a specific isolate of *C. globosum* could inhibit *Pyricularia oryzae*, the causative agent of rice blast disease (Soytong & Quimio, 1989). After 30, 45, and 60days of nano-CGH, nano-CGE, and nano-CGM treatments, the plant height and tiller number in pot experiments were increased, And after 60days the nano CGH given the best overall effect, as shown in Tables 3 and 4.

## CONCLUSION

The nanoparticles containing *C. globosum* KMITL-N0805 extracts were yellowish in color,with a particle size of 241 nm. Nano-CGH, nano-CGE, and nano-CGM from *C. globosum* KMITL-N0805 expressed antifungal activity against *C. lunata* with $ED_{50}$ values of 1.21, 1.19, and 1.93 ppm/mL, respectively. It was concluded that nano-CGH, nano-CGE, and nano-CGM from *C. globosum* KMITL-N0805 were effective in decreasing the leaf spot disease of rice var. Sen Pidoa caused by *C. lunata.* They also increased the plant height and tiller number significantly, when compared to the non-treated control. This is the first report on nanoparticles loaded with extracts derived from *C. globosum* KMITL-N0805 with the ability to control leaf spot of rice caused by *C. lunata*.

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## REFERENCES

Aggarwal, R., Tewari, A.K., Srivastava, K.D. & Singh, D.V. 2004. Role of antibiosis in the biological control of spot blotch (*Cochliobolussativus*) of wheat by *Chaetomium globosum*. *Mycopathologia*, **157**: 369-377.

Ditta, A. 2012. How helpful is nanotechnology in agriculture? *Advances in Natural Sciences: Nanoscience and Nanotechnology*, **3**: 033002.

Dar, J. & Soytong, K. 2014. Construction and characterization of copolymer nanomaterials loaded with bioactive compounds from *Chaetomium* species. *Journal of Agricultural Technology*, **10(4)**: 823-831.

Dhingra, O.D. & Sinclair, J.B. 1987. *Basic Plant Pathology Methods*. 1st ed. CRC Press, Florida. 355 pp.

Elibol, O.H., Morisette, D.D., Denton, J.P. & Bashir, R. 2003. Integrated nanoscale silicon sensors using top-down fabrication. *Applied Physics Letters*, **83**: 4613-4615.

Kanokmedhakul, S., Kanokmedhakul, K., Phonkerd, N., Soytong, K., Kongsaeree, P. & Suksamrarn, A. 2002. Antimycobacterialantraquinone-chromanone compound and diketopiperazine alkaloid from the fungus *Chaetomium globosum* KMITL-N0802. *Planta Medica*, **68**: 834-836.

Liu, Z.H., Yang, Q., Hu, S., Zhang, J.D. & Ma, J. 2008. Cloning and characterization of a novel chitinase gene (*chi*46) from *Chaetomium globosum* and identification of its biological activity. *Applied Microbiology and Biotechnology*, **80**: 241-252.

Li, M., Huang, Q. & Wu, Y. 2011. A novel chitosan-poly (lactide) copolymer and its submicron particles as imidacloprid carriers. *Pest Management Science*, **67**: 831-836.

Nguyen, H.M., Hwang, I.C., Park, J.W. & Park, H.J. 2012. Enhanced payload and photo-protection for pesticides using nanostructured lipid carriers with corn oil as liquid lipid. *Journal of Microencapsulation*, **29**: 596-604.

Park, J.H., Choi, G.J., Jang, K.S., Lim, H.K., Kim, H.T., Cho, K.Y. & Kim, J.C. 2005. Antifungal activity against plant pathogenic fungi of chaetoviridins isolated from *Chaetomium globosum*. *FEMS Microbiology Letters*, **252**: 309-313.

Perlatti, B., de Souza Bergo, P.L., das Graças Fernandes da Silva, M.F., Fernandes, J.B. & Forim, M.R. 2013. Polymeric nanoparticle-based insecticides: a controlled release purpose for agrochemicals. In: *Insecticides – Development of Safer and More Effective Technologies*. S. Trdan (ed.). In Tech Open Access, Croatia. Ch. 20.

Rai, M. & Ingle, A. 2012. Role of nanotechnology in agriculture with special reference to management of insect-pest. *Applied Microbiology and Biotechnology*, **94(2)**: 287-293.

Salata, O.V. 2004. Application of nanoparticles in biology and medicine. *Journal of Nanobiotechnology*, **2**: 3.

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

Soutter, W. 2012. Nanotechnology in agriculture. AZoNano.com Publishers. Available at http://www.azonano.com/article.aspx?ArticleID=3141#1%204. Accessed on May 25, 2013.

Soytong, K. & Quimio, T.H. 1989. Antagonism of *Chaetomium globosum* to the rice blast pathogen, *Pyricularia oryzae*. *Kasetsart Journal (Natural Science)*, **23**: 198-203.

Soytong, K., Kanokmedhakul, S., Kukongviriyapa, V. & Isoba, M. 2001. Application of *Chaetomium* (Ketomium®) as a new broad spectrum biological fungicide for plant disease control: a review article. *Fungal Diversity*, **7**: 1-15.