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## Construction and characterization of copolymer nanomaterials loaded with bioactive compounds from *Chaetomium* species

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The need to develop safe, economical and effective methods to control disease in crop production is deemed necessary. Several researches had proven that crude extracts from *Chaetomium* species can control plant pathogens. Incorporation of drugs into nanofibers using polylactic acid and electrospinning was also reported to be successful. This research aimed to develop and characterize nanomaterial loaded with active compounds from *Chaetomium* species. Methanol crude extract from *Chaetomium globosum* and *Chaetomium cupreum* were used in this study. The extracts were incorporated into polylactic acid and electropun at 25-30 kV. Results show that the product from *C. globosum* had yellowish color while the one from *C. cupreum* had plae orange color. Scanning electron microscope images revealed that the nanomaterial from *C. globosum* measured 241 nanometers, which is higher than in *C. cupreum*, with only 171 nanometers. FTIR analysis showed that the nanomaterial from *C. globosum* had more peaks than in *C. cupreum*.

**Keywords:** *Chaetomium*, crude extract, nanomaterial, electrospinning

### Introduction

About 126 million cases and 220,000 people die each year due to poisoning from toxic chemicals being used as agricultural inputs to control pest and diseases in crops (WHO and UNEP as cited by Richter, 2002). As such the need to find alternative ways which are safe both to human and environment as well as effective and efficient methods to control natural pests of plant or crops is hereby needed. One of the best methods nowadays is the use of nanotechnology. Nanotechnology is the building, re-structuring, controlling and devising materials at the molecular level. "Nano" came from the root word nanometer. A nanometer (nm) is one-billionth of a meter. There are two possible approaches to nanotechnology: 'top-down' and 'bottom-up'. 'Top-

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down' refers to making nanoscale structures, for example photonic applications in nanoelectronics and nanoengineering. On the other hand, 'bottom-up', or molecular nanotechnology, applies to building organic and inorganic materials into defined structures, atom by atom or molecule by molecule, often by self-assembly or self-organization. Applications of nanotechnology had advanced greatly, from basic modeling machines, computer microchips, fiber optics and now to pharmacology, medicine and food industries (Li, *et al.*, 2011). Biologists and chemists are actively engaged in the synthesis of inorganic, organic, hybrid and metal nanomaterials including different kinds of nanoparticles having unusual properties like optical, physical, biological and so on (Elibol *et al.*, 2000; Salata, 2004). Nanocarrier systems provide stability to compounds that are otherwise sensitive to conditions including ultraviolet light (UV) or oxidation (Anton *et al.*, 2008) and control the release rate of incorporated compounds (Liu, *et al.*, 2009).

Currently, the possible uses of nanotechnology in agriculture are being explored. Precision farming, for example, along with nano-delivery systems are becoming the new "industrial revolution" in agriculture (Soutter, 2012). Precision farming enables farmers to maximize crop production while utilizing minimum farm inputs especially inorganic chemicals as pesticides and fertilizers. The use of micro-sensors and other automatic monitoring devices that can provide up to date or even up to minute reports about crop or animal conditions will surely help farmers improve their farming system thus increase yield and income. As such, there is great potential for nanoscience and technology in giving state-of-the-art solutions for various challenges faced by agriculture and society today (Ditta, 2012). Nanoparticles can serve as 'magic bullets', containing herbicides, chemicals, or genes, which target particular plant parts to release their content. They can also enable effective penetration of chemicals through cuticles and tissues, allowing slow and constant release of the active substances. The most popular shapes of nanomaterials being used for biocides delivery are: Nanospheres, nanocapsules, nanogels (Perlatti *et al.*, 2013).

The potential uses and benefits of nanotechnology are enormous. These include insect pests management through the formulations of nanomaterials-based pesticides and insecticides, enhancement of agricultural productivity using bio-conjugated nanoparticles (encapsulation) for slow release of nutrients and water, nanoparticle-mediated gene or DNA transfer in plants for the development of insect pest-resistant varieties and use of nanomaterials for preparation of different kind of biosensors, which would be useful in remote sensing devices required for precision farming. Traditional strategies like integrated pest management used in agriculture are insufficient, and application

of chemical pesticides like DDT have adverse effects on animals and human beings apart from the decline in soil fertility. As a result, nanotechnology would provide green and efficient alternatives for the management of insect pests in agriculture without compromising nature (Rai and Ingle, 2012). Consequently, nanotechnology has great potential in agriculture because it can enhance the quality of life through its applications especially in the food system. It may play a very important role in the development of any nation but we should be careful with any new technology to be introduced. Based on this assumption, there is a need to inform the public about its advantages; this may result to an increase in interest and new applications yet to be discovered (Ditta, 2012).

Some nanoparticles have been formulated containing pesticides in colloidal suspensions or powder, in nano or micro scale. These preparations have advantages such as increasing stability of the active organic compound (UV, thermal, hydrolysis, etc.), foliar settling, reduction in foliar leaching, systemic action, synergism, specificity and so on (Perlatti *et al.*, 2013). As consequence, the amount of insecticide necessary (dosage), the number of applications, human exposure to insecticides and environmental impact are reduced. The nano- and microformulations have been employed not only for synthetic insecticides but also in alternative products to control plague insects such as natural products (herbal extracts) and microorganisms.

The today's issues like climate change, urbanization, sustainable use of natural resources, runoff and accumulation of toxic pesticides, herbicides and fertilizers need to be addressed immediately (Ditta, 2012). However, only a few studies have reported the use of nanocarrier systems in agriculture (Nguyen *et al.*, 2012). Bio-active compound from different *Chaetomium* species has been proven by Soyong *et al.* (2001; 2013) and Sibounnavong *et al.* (2012) to be an effective antifungal agent against several plant pathogens. Inasmuch as the quest for safe, effective, environmentally friendly methods of controlling plant pathogen is highly desired, the construction and characterization of copolymer nanoparticle loaded with bio-active compound and not toxic pesticide is need, thus this research.

This research aimed to construct and characterize copolymer nanoparticle that carried bioactive compounds from crude extract of *Chaetomium* species. Specifically: prepared nano-particles using electrospinning technique; viewed the particles using scanning electron microscope; measured and compared the sizes of the nanoparticles; and characterized using Fourier Transform Infrared spectroscopy.

## **Materials and methods**

### ***Preparation and extraction of crude extract (bioactive compounds)***

Pure cultures of *Chaetomium globosum* and *Chaetomium cupreum* (courtesy of Assoc. Prof. Dr. Kasem Soyong, KMITL) were subcultured in PDA. Approximately 1000 petri dishes containing 25 mL potato dextrose broth were prepared and 1x1 mm potato dextrose agar with pure culture of the fungus was transferred to each petri dish. Each species of fungus was cultured separately. The plates were incubated at room temperature for about 1 month or until the surface of the plate was covered by the fungus. The mycelia mats were harvested and air dried. Crude extracts from antagonistic fungi was done using the method of Kanokmedhakul *et al.* (2006). The fungal biomass was ground with electrical blender and then soaked in hexane for five days, then filtered and the filtrate was evaporated using rotary vacuum evaporator. The marc was soaked in ethyl acetate, the same procedure was done as with hexane and finally methanol.

### ***Preparation and characterization of nano-particles***

The extract was made into nano-particle by electrospinning. Exactly 2 grams of polylactic acid (PLA) was dissolved in 10 mL tetrahydrofuran. The mixture was heated until the polylactic acid was totally melted. The extract was dissolved in a few drops of dimethylsulfoxide and heated to dissolve completely and then the two mixtures were added together. The resulting mixture was loaded into a syringe and placed into the electrospinning set-up. The tip of the syringe was clipped with the positive pole while the aluminum foil was clipped with the negative pole and serves as the collector. The voltage used was 25 to 30 kilovolts. Nano-particle containing no active compound was also made and served as the control. The product was carefully scraped from the aluminum foil and stored in tightly capped bottles.

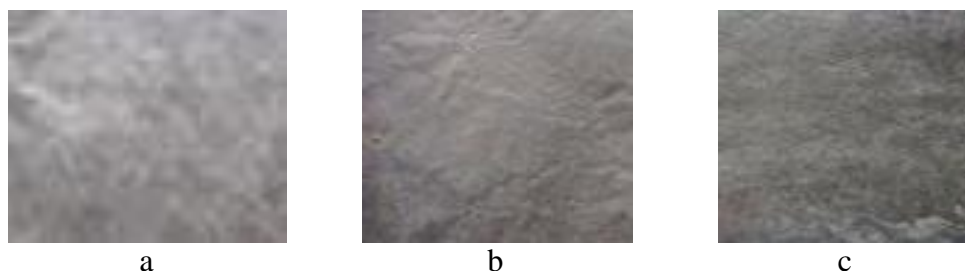
The characteristics of the products were noted, viewed under the scanning electron microscope and the properties were analyzed using Fourier Transform Infrared spectroscopy.

## **Results and discussions**

The appearance of the electrospun materials were first noted through visual observation by the naked eye. It was observed that the control (PLA alone) has white color. The PLA containing *C. globosum* extract has pale

yellow color while the one with *C. cupreum* extract is very light orange. The difference in color is attributed to the pigments present in the extract (Fig. 1).

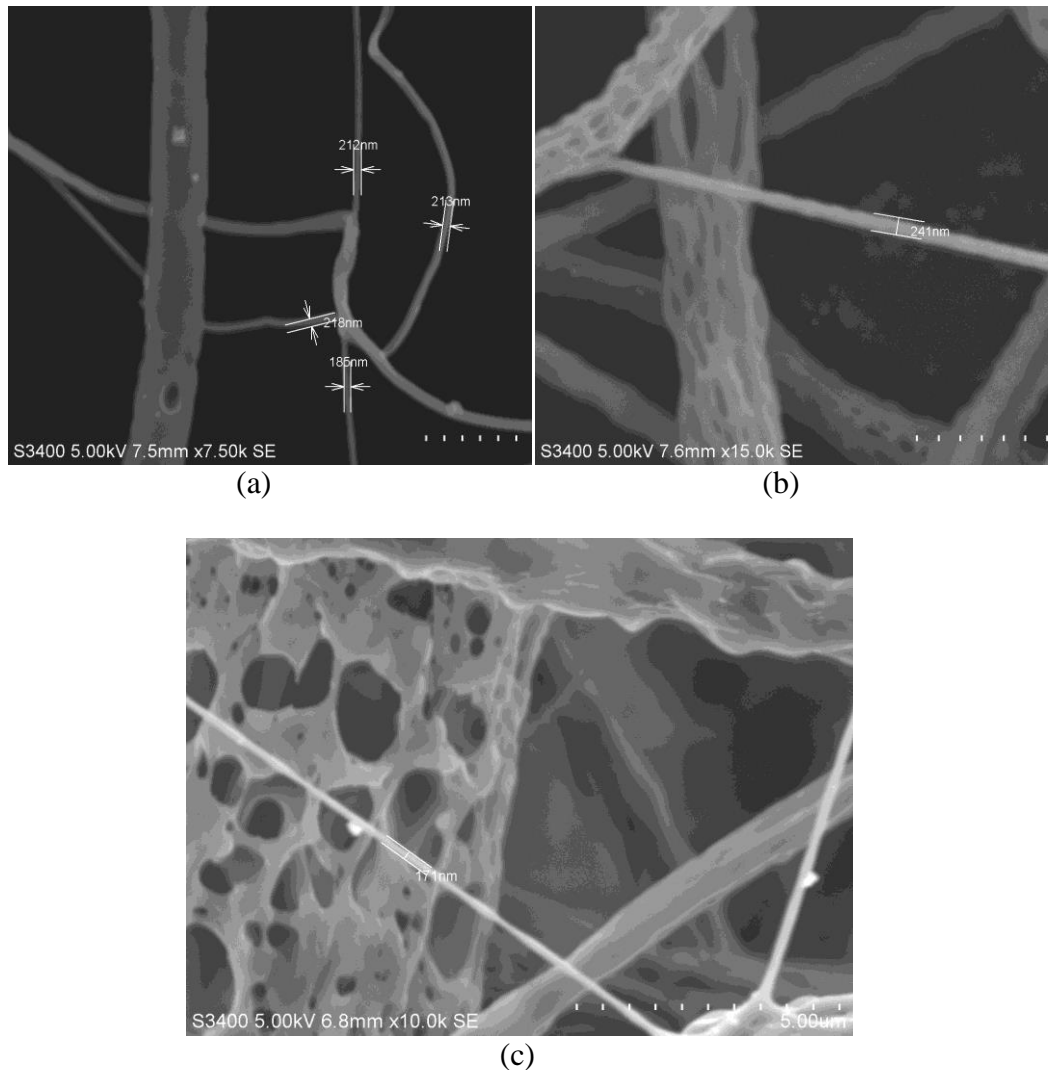
The particle size in the control (PLA alone) ranged from 185-218 nanometers while in *C. globosum* was 241 nanometers and in *C. cupreum* was 171 nanometers (Fig. 2). It could be observed that in the control, the fibers are long and do not tangle with each other as compared to the fibers containing extract from *C. globosum* and *C. cupreum*. The presence of hollows or holes in the fibers containing extracts indicates formation of strong bonds, making the fibers more entangled.



**Fig. 1** Physical appearance of the products after electrospinning. (a) control- PLA alone (b) extract from *C. globosum* and (c) extract from *C. cupreum*

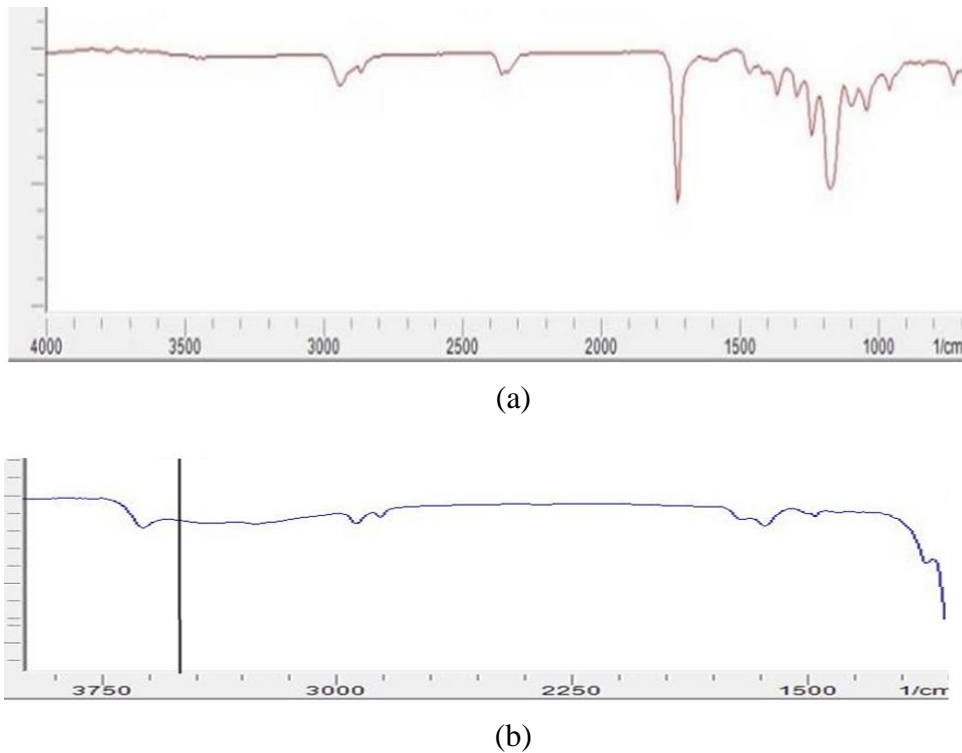
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The high surface-to-volume ratio together with size effects (quantum effects) of nanoparticles has many size-dependent phenomena such as biological, chemical, electronic, magnetic and mechanical properties. For example, the melting point of nanoparticles is evidently decreased when the size is reached to the nanometer scale (Nalwa, 2004 and Wang, 2000). The particle size plays a crucial role in nanoparticle properties and therefore an essential task in property characterization of nanoparticles is particle sizing (Akbari1 *et al.*, 2011).



**Fig. 2.** Scanning electron micrograph of the nano-particles. (a) control- PLA alone (b) extract from *C. globosum* and (c) extract from *C. cupreum*.

The result of Fourier Transform Infrared spectroscopy revealed that the nanomaterial from *C. globosum* had more peaks than in *C. cupreum* (Fig. 3). This indicates that nanomaterial with *C. globosum* extract had more stretches, wags and bends in the developed product as compared to the one containing *C. cupreum* extract. This finding coincides with the result on the size of the products wherein the nanomaterial with *C. globosum* extract measured larger than nanomaterial with *C. cupreum* extract.



**Fig. 3.** FTIR analysis of the nanomaterials (a) PLA with *C. globosum* extract (b) PLA with *C. cupreum* extract

According to Wei *et al.* (2012) in their study about incorporating captopril into PLA, FTIR analysis revealed that the process of electrospinning changed the physical form of Captopril, but its chemical structure remained unchanged (Wei *et al.*, 2012). This imply that since electrsopinning does not affect the chemical structure of the active compounds, this may also imply that the active compound may had retained its biological activity.

### Conclusion

Methanol crude extract from *Chaetomium globosum* and *Chaetomium cupreum* were used in this study. The extracts were incorporated into polylactic acid and electropun at 25-30 kV. Results show that the product from *C. globosum* had yellowish color while the one from *C. cupreum* had plae orange color. Scanning electron microscope images revealed that the nanomaterial from *C. globosum* measured 241 nanometers, which is higher than

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## Recommendations

Further study should be conducted to determine the affectivity of the developed product against plant pathogens both in *in-vitro* and *in-vivo* studies. In depth research on the effect of these developed nanomaterials on plant immunity such as detection of phytoalexins should also be studied.

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