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# Nanofibers derived from endophytic *Chaetomium brasilense* for growth stimulation of rice

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**Abstract** *Chaetomiumn brasilense* PT302 was endophytically isolated from rice var Supanburi 1 and Prathumthani 80 which firstly selected for investigation to promote the growth of rice. It is morphologically and molecular phylogenetic confirmation through ITS1 and ITS4 as *Ch.brasilense* PT302 compared to *Achaetomium strumarium* (JX863914) as out group. Crude metabolites **(**crude hexane, crude ethyl acetate and crude methanol) at 50 ppm from *Ch. brasiliense* were proved to promote seed germination of rice var. Supanburi 1 and Pathumtani 80 at 7 days which found significantly higher seed germination than the non-treated control. Nanofibers constructed *Ch. brasiliense* promoted significantly higher seed germination, plant height and number of tillers than the non-treated control in rice var Supanburi 1 and Prathumthani 80 in 7 days at low concentration of 5 ppm planted in Chachengsao and Bangkok soil series. Nanofibers derived from endophytic *Ch. brasilense* is actively promoted plant growth at lower concentration than crude metabolites. It is firstly reported the natural product nanofiber constructed from endophytic *Ch. brasilense* to stimulate plant growth.

**Keyword:** Rice, Nanofibers, Plant growth promotion, Chaetomium

## Introduction

Agricultural nanotechnology is increasingly interested in agriculture (Li *et al.,* 2011b). The organic nanoparticles are unusual properties with physical and biological ones (Elibol *et al.,* 2000). Nanotechnological application in agriculture are investigated by many researchers (Soutter, 2012). Endophytic fungi are able to produce mixture of volatile organic compounds that are lethal to human, plant pathogenic fungi and bacteria. They are reported to be associated with plants as symbiotic relation. Some endophytic fungi are stated to associate with plants that confer to pathogenic resistance. Endophytic *Chaetomium globosum* is found to be antifungal metabolites in Gingkgo(Qin *et al.*, 2009 and Li *et al.*, 2011a). *Chaetomium cochliodes* is reported to control brown leaf spot of rice (Soytong, 2014).

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Endophytic fungi from rice (*Oryza sativa* L.) is reported to isolate many fungi including *Chaetomium brasilense*, *Chaetomioum cupreum* and *Chaetomium globosum* (Leewijit *et al.*, 2016). Khumkomkat *et al.* (2009) found that *Chaetomium brasiliense* produces active compounds which actively antimalarial and cytotoxic depsidones.

Previous study found endophytic fungi in many plants resulted to be *Nigrospora sphaerica* from *Chrysalidocarpus lotescens* and *Fusarium falciforme* from *Mascarena lagencuulis*. Crude metabolites from those endophytic fungi are actively tested against *Colletotrichum coffeanum* causing coffee anthracnose (Song *et al.*, 2016). Song *et al.* (2018) found that nanoparticles derived from *Chaetomium elatum* showed the highest inhibition *Pyricularia oryzae* causing rice blast and seem to be promoted plant growth. Song *et al* (2021) found that application of antifungal nanomaterials from *Emericella nidulans* gave a good control rice blast and tended to increase plant growth parameters. The objective was to evaluate nanofibers constructed from endophytic *Ch. brasiliense* from rice to promote plant growth of rice var Supanburi 1 and Prathumthani 80 in Chachengsao and Bangkok soil series.

## Materials and methods

### *Isolation of endophytic Chaetomium from rice var. Supanburi 1 and Prathumthani 80*

The healthy rice seedling samples var Supanburi 1 and Prathumthani 80 were surface disinfected with Clorox 10 % and cleaned with sterilized water, then cut the leaves, stem and roots and removed the epidermis tissues , cut to 3X3 $mm^3$ , and placed to water agar (WA) and transferred to get pure cultures. Endophytic isolate was isolated from rice (*Oryza sativa* L.) according to method of Song *et al.* (2016) and morphological identification by culturing on potato dextrose agar (PDA) , incubated at room temperature (28-30 C) for 21 days. It was observed under binocular microscope to measure fruiting structures, terminal and lateral hairs, asci and ascospores according to Domsch *et al*. (1993) and Ellis (1971).

### *Nucleotide sequencing by DNA extraction*

The isolate was cultured in potato dextrose broth (PDB) for 3 days at 24 C. DNA was extracted using CTAB (cetyl trimethyl ammonium bromide) which modified from Marc and Zhang (1999). The mycelia were washed with 25 µl EDTA, centrifuged at 6,000 rpm, ground with liquid nitrogen and put into tube with added 1.5 mL 2X CTAB buffer of 700 ml, incubated at 65 C for 1-2 h, shaking for 2 min. ,then added chloroform: Isoamyl alcohol at the ration of 24:1 in 700 ml., and centrifuged at 14,000 rpm. For 5 min.at 4 C. The solution in 1.5 ml was added RnaseA at 20 mg/ml, incubated at 37 C for 30 min and added 10%CTAB of 50 ml incorporated with Chloroform: Isoamyl alcohol at ration of 24: 1 in 700 ml., centrifuged at 14000 rpm for 15 min. The solution of 1.5 ml was added isopropanol 400-500 µl at 20 C for 30 min. then centrifuged at 14,000 rpm/min for 15-30 min. DNA was washed with 70 % ethanol in 1000 µl for 3min, centrifuged at 14000 rpm/min, removed ethanol and washed again with 95% ethanol, centrifuged at 14,000 rpm/min for 30 min, then removes ethanol, incubated at 37 C for 20 min to evaporate ethanol, and dissolved with 20 µl TBE buffer incubated at 37 C for 2-3 h. DNA was duplicated by polymerace chain reaction (PCR) using ITS (Internal Transcribed Spacer) with primer ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.,* 1990). PCR product was done for nucleotide sequence and compared to GenBank using Basic Local Alignment Search Tool (BLAST) and ClustalW2, analysed PhylogeneticTree with compared to Mega7program (Neighbor-joiningmethod,NJ).

***Nanofibers and crude metabolites from endophytic Chaetomium brasiliense to promote the growth of rice var. Supanburi 1 and Prathumthani 80***

Nanofibers derived from active crude metabolites of *Ch. brasiliense,* crude hexane, crude ethyl acetate, and crude methanol namely nano-CBH, nano-CBE and nano-CBM incorporated at the concentrations of 0, 1, 3, 5, 7, 9, and 10 ppm which were tested for promoting plant growth of rice var. Supanburi 1 and Prathumthani 80.

Crude metabolites of *Ch. brasiliense*, crude hexane (CBH), crude ethyl acetate (CBE), and crude methanol (CBM) were tested to promote plant growth of rice var. Supanburi 1 and Prathumthani 80 at the concentrations of 0, 10, 50, 100, 500 and 1000 ppm using poisonous medium technique. The experiment was performed using factorial in Completely Randomized Design (CRD) with four replications. Data were collected as germination, plant height, root length.

***Endophytic Chaetomium brasilense for promoting the growth of rice var Supanburi 1 and Prathumthani 80 in pot experiments***

The experiment was arranged in RCBD with four replications. Two experiments were done in Chachengsao and Bangkok soil series. Treatments were set up as follows: non-treated control (T1), treated with crude mixture of CB (T2), nano-CB and spore suspension of CB. 15 days of rice seedlings var. Supanburi 1 and Prathumthani 80 were planted into pots containing sterilized soil in each treatment. Data were collected as plant height (cm) and tillers. Experimental designs were arranged in Randomized Completely Block Design (RCBD), and data were statistical computed analysis of variance (ANOV). The averaged means in each treatment were compared using Duncan's Multiple Range Test (DMRT) at P=0.05 and 0.01.

## Results

### *Isolation of endophytic Chaetomium from rice*

*Ch. brasilense* PT302, *Ch. cupreum* SP101 and *Ch. globosum* 01 were isolated from the healthy rice seedling samples var Supanburi 1 and Prathumthani 80. Those isolates were basically morphology and molecular identified. *Ch. brasilense* (PT302) was firstly selected for further experiment (Figure 1).

**Figure 1.** *Chaetomium brasiliense,* A = culture on PDA for 30 days, B= Ascocarps (100X), C= Asci (400X), D = Ascospores (400X)

### *Nucleotide sequencing by DNA extraction*

*Chaetomium* spp isolates PT302, PT301 and SP101 was molecular phylogenic confirmation by nucleotide sequencing on Nuclear Ribosomal DNA (rDNA). DNA was multiplied in rDNA at Internal Transcribed Spacer (ITS) on the position of ITS1-5.8S-ITS2 by primers of ITS1and ITS4. It found that all isolates appearing at 400-500 bases. The analysis was compared nucleotide sequencing by Mega 7 (Kumar *et al*., 2016) and GenBank found that isolate PT302 similarity to the isolates of JX966545, JX536279, FR718872, KX146504, KT357687, KT357683, KT357682, KT357646 and KF680267 at 97 % to *Ch.brasilense*. The phylogenic tree was compared to GenBank by Neighbour-Joining showed that biodiversity of the fungal endophytes related to morphology and nucleotide sequences at ITS region ITS of *Chaetomium* sp. It can be arranged which closely related to four groups, A was *Ch.globosum* 01, group B was *Ch.brasilense* PT302, and group C was *Ch. cupreum* SP101 which distinguished to group D as *Achaetomium strumarium* (JX863914) was out group (Figure 2).

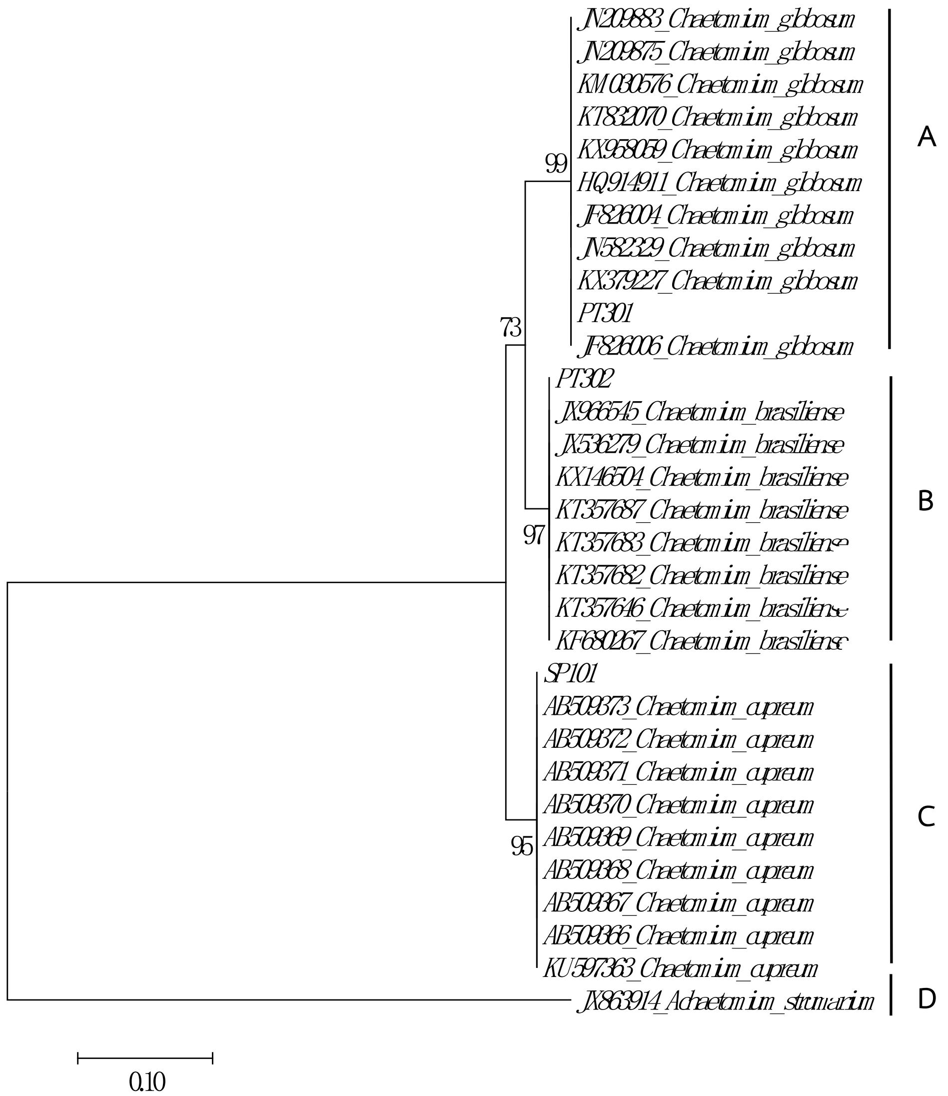


**Figure 2.** Phylogenetic tree of *Chaetomium* spp. which performed by PCR at ITS region and evaluation relationship analysis of neighbor-joining method with 1000 times bootstrap using MEGA 7

***Bioactive metabolites from endophytic Chaetomium brasilense***

**Extraction**

The crude metabolites of *Ch. brasilense* was extracted by following the method of Kanokmedhakul *et al.* (2006). Each isolate was cultured in PDB for 30 days, filtered, dried for 7 days at room temperature to be fungal biomass. Dried biomass was weight 190, 90.96 and 133.03 g, respectively. It was ground in electrical blender, and seriously soaked for 5 days in Hexane, Ethyl acetate and Methanol respectively. They were then separated the marc and filtrate. Filtrate was passed through Rotary vacuum evaporator. Biomass of *Ch. brasilense* yielded crude Hexane, Ethyl acetate and Methanol 0.758, 4.149 and 5.117g. These crude extracts were then constructed through electron spinning machine to be nano-particles according to the method of Dar and Soytong (2014). Nano-particles are shown in Figure 3.

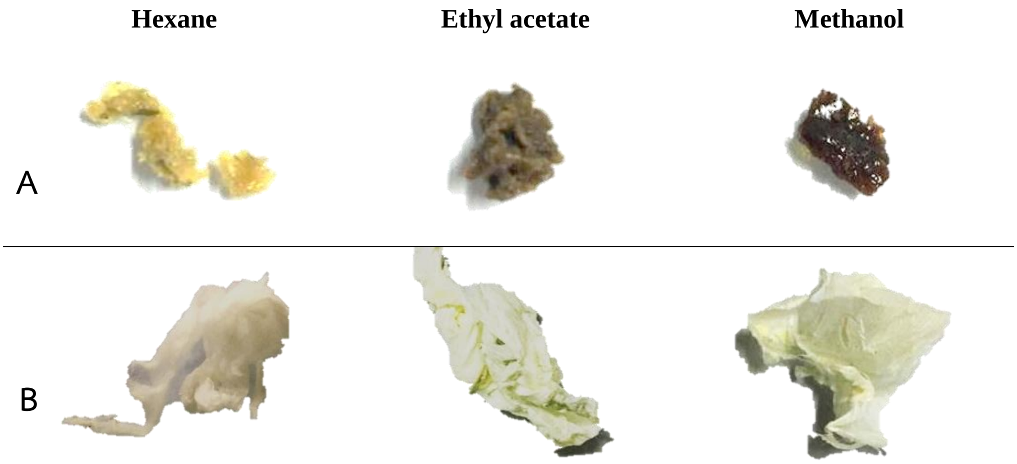


**Figure 3.** Crude metabolites from endophytic Chaetomium spp A = *Ch. brasilense,* B = nanoparticles *Ch. brasilense*

**Crude metabolites from endophytic Chaetomium brasiliense to promote the growth of rice var. Supanburi 1 and Prathumthani 80**

Crude metabolites **(**crude hexane, crude ethyl acetate and crude methanol) from *Ch. brasiliense* were tested for seed germination of rice var.Supanburi 1 at 7 days found that crude ethyl acetate at 50 ppm gave significantly highest seed germination of 72.5 %, and followed by crude hexane and crude methanol at 500 and 50 ppm which were 70 and 67.5 %, respectively when compared to the non-treated control. Crude metabolites from *Ch. brasiliense* were significantly promoted the plant height and root length of rice var. Supanburi 1 which crude hexane at 100 ppm showed the highest plant height and roots of 21.23 and 19.78 mm., respectively, and followed by crude methanol at 100 ppm which were 16.31and 19.45 mm., respectively when compared to the non-treated control. Result showed that crude hexane, crude ethyl acetate and crude methanol of *Ch. brasiliense* affected germination of rice seeds var Pathumtani 80 which revealed that crude hexane of *Ch. brasiliense* at 50 ppm gave highest seed germination of 72.5 % when compared to non-treated control, and followed by crude methanol and crude ethyl acetate at 50 and 100 ppm as 67.5 and 70 %, respectively. The tested highest concentration at 1000 ppm gave the lowest seed germination. Crude methanol of *Ch. brasiliense* at 100 ppm gave the highest plant height and root length as 22.33 and 22.86 mm. respectively, and followed by crude ethyl acetate at 100 ppm resulting plant height and root length of 21.18 and 22.64 mm, respectively when compared to the non-treated control (Table 1, 2).

**Nanofibers derived from endophytic Chaetomium brasiliense to stimulate the growth of rice var Supanburi1 and Prathumthani 80**

Nanofibers constructed from crude hexane, crude ethyl acetate and crude methanol of *Ch. brasiliense* affected seed germination of rice var Supanburi 1 in 7 days at low concentration. Nano-CBH at 5 ppm gave the highest seed germination of 72.5 % and followed by Nano-CBM and Nano-CBE at 3 and 5 ppm as 67.5 and 60 %., respectively when compared to the non-treated control. Nano-particles at 10 ppm gave the lowest seed germination. Nano-CBH of *Ch. brasiliense* affected the highest plant height and root length at 5 ppm which were 20.43 and 23.71 mm., respectively and followed by Nano-CBE at 3 ppm were 19.81 and 20.64 mm., respectively when compared to the non-treated control. Nano-CBH derived from *Ch. brasiliense* at 3 ppm gave the highest seed germination of rice var Pathumthani 80 in 7 days resulted seed germinatioin of 72.5 % and followed by Nano-CBM and Nano-CBE at 5 ppm which were 70 %. Nano-CBE constructed from *Ch. brasiliense* at 5 ppm showed the highest in plant height and root length of 18.30 and17.60 mm., respectively and followed by Nano-CBM at 5 ppm which were 17.80 and 16.22 mm., respectively.

**Table 1.** Crude Extracts of *Ch. brasiliense* for growth stimulant of rice var Supanburi 1 in 7 days

| Crude extracts | conc (ppm) | germination (%) | Plant height (mm) | Root length (mm) |
|---|---|---|---|---|
| CBH | 0 | 45 | 12.72f1[1/] | 9.79de[1/] |
| | 10 | 25 | 16.37bc | 13.35b-d |
| | 50 | 52.5 | 17.58b | 15.81a-c |
| | 100 | 65 | 21.23a | 19.78a |
| | 500 | 70 | 9.19g | 10.10de |
| | 1000 | 27.5 | 5.17h | 5.67f |
| CBE | 0 | 45 | 12.32f | 9.73de |
| | 10 | 67.5 | 15.39b-e | 12.29cd |
| | 50 | 72.5 | 13.04ef | 16.02a-c |
| | 100 | 42.5 | 12.32f | 10.27de |
| | 500 | 60 | 12.69f | 10.38de |
| | 1000 | 30 | 6.02h | 4.65f |
| CBM | 0 | 65 | 12.77f | 10.29de |
| | 10 | 60 | 13.67d-f | 14.55bc |
| | 50 | 67.5 | 15.52b-d | 13.70b-d |
| | 100 | 65 | 16.31bc | 19.45a |
| | 500 | 65 | 13.98c-f | 17.50ab |
| | 1000 | 45 | 9.94g | 6.97ef |
| | C.V. (%) | | 11.75 | 21.46 |

[1]Averages of four repeated experiments which followed by a common letter were not significantly differed by DMRT at P 0.01.

**Table 2.** Crude Extracts of *Ch.brasiliense* for growth stimulant of rice var. Pathumthani 80 in 7 days

| Crude extracts | conc (ppm) | germination (%) | Plant height (mm) | Root length (mm) |
|---|---|---|---|---|
| CBH | 0 | 65 | 12.41f-h[1/] | 11.86f-h[1/] |
| | 10 | 57.5 | 17.02cd | 17.82b |
| | 50 | 72.5 | 20.21ab | 21.01a |
| | 100 | 67.5 | 14.68d-f | 16.80b-d |
| | 500 | 42.5 | 11.14g-i | 14.23d-f |
| | 1000 | 40 | 8.96i | 9.71hi |
| CBE | 0 | 62.5 | 13.17e-g | 11.94f-h |
| | 10 | 57.5 | 16.60c | 14.44c-f |
| | 50 | 52.5 | 19.03bc | 16.95bc |
| | 100 | 70 | 21.18ab | 22.64a |
| | 500 | 42.5 | 17.16cd | 15.34b-e |
| | 1000 | 40 | 9.39i | 9.25i |
| CBM | 0 | 62.5 | 13.02e-g | 11.34g-i |
| | 10 | 52.5 | 16.35cd | 14.12ef |
| | 50 | 57.5 | 18.53bc | 15.32b-e |
| | 100 | 67.5 | 22.33a | 22.86a |
| | 500 | 62.5 | 15.15de | 13.62e-g |
| | 1000 | 40 | 10.04hi | 10.09hi |
| | C.V. (%) | | 11.36 | 11.09 |

[1]Averages of four repeated experiments which followed by a common letter were not significantly differed by DMRT at P 0.01.

**Table 3.** Nanofibers of *Ch.brasiliense* for growth stimulant of rice var Supanburi 1 in 7 days

| (Nano-particles) | concentration (ppm) | germination (%) | Plant height (mm) | Root length (mm) |
|---|---|---|---|---|
| Nano-CBH | 0 | 70 | 11.54fg[1/] | 11.42def[1/] |
| | 1 | 55 | 18.53a-c | 16.62c |
| | 3 | 60 | 19.25ab | 22.36ab |
| | 5 | 72.5 | 20.43a | 23.71a |
| | 7 | 65 | 17.76a-c | 13.54 c-e |
| | 10 | 52.5 | 9.78g | 9.96fg |
| Nano-CBE | 0 | 70 | 11.00fg | 9.32fg |
| | 1 | 65 | 11.40fg | 13.64c-e |
| | 3 | 52.5 | 19.81ab | 20.64ab |
| | 5 | 60 | 17.19bc | 14.07cd |
| | 7 | 50 | 14.11de | 9.93fg |
| | 10 | 45 | 10.13g | 4.65h |
| Nano-CBM | 0 | 70 | 10.94fg | 15.55c |
| | 1 | 60 | 14.37de | 19.62b |
| | 3 | 67.5 | 18.87a-c | 19.67ab |
| | 5 | 55 | 16.19cd | 10.49e-g |
| | 7 | 62.5 | 13.07ef | 10.12fg |
| | 10 | 42.5 | 8.94g | 7.75g |
| | C.V. (%) | | 11.77 | 14.83 |

[1]Averages of four repeated experiments which followed by a common letter were not significantly differed by DMRT at P 0.01.

**Table 4.** Nanofibers of *Ch.brasiliense* for growth stimulant of rice var Pathumthani 80 in 7 days

| (**Nano-particles)** | **Conc. (ppm)** | **germination (%)** | **Plant height (mm)** | **Root length (mm)** |
|---|---|---|---|---|
| Nano-CBH | 0 | 67 | 9.79g[1/] | 10.77g[1/] |
| | 1 | 72 | 12.33ef | 15.57c-e |
| | 3 | 72.5 | 17.14ab | 19.02a |
| | 5 | 60 | 14.28c-e | 18.97a |
| | 7 | 50 | 13.08e | 18.16ab |
| | 10 | 42.5 | 9.95g | 11.5fg |
| Nano-CBE | 0 | 67 | 10.77fg | 10.78 |
| | 1 | 67.5 | 13.97c-e | 13.95de |
| | 3 | 60 | 15.63b-d | 14.54de |
| | 5 | 70 | 18.30a | 17.60a-c |
| | 7 | 55 | 13.88c-e | 13.58ef |
| | 10 | 40 | 9.65g | 10.34g |
| Nano-CBM | 0 | 67 | 10.52fg | 10.54g |
| | 1 | 40 | 13.48de | 15.10de |
| | 3 | 55 | 15.73b-d | 14.42de |
| | 5 | 70 | 17.80ab | 16.22b-d |
| | 7 | 67.5 | 15.91bc | 13.67ef |
| | 10 | 42.5 | 9.76g | 8.04h |
| | C.V. (%) | | 10.68 | 10.91 |

[1]Averages of four repeated experiments which followed by a common letter were not significantly differed

[1]Averages of four repeated experiments which followed by a common letter were not significantly differed by DMRT at P 0.01.

Chachengsao and Pathumthani soil seriers were compared by analysis for organic matter, pH, total nitrogen (N), and phosphorous (P) and potassium (K). Chachengsao soil. series before planting showed soil pH, organic matter, N, P and K as 5.65, 3.28 %, 0.16 %, 12.8 ppm , 578 ppm., respectively. Pathumthani soil series before planting found soil pH, organic matter, N, P and K of 4.67, 3.07%, 0.15 %, 7.16 ppm ,242 ppm, respectively.

### *Endophytic Chaetomium brasilense for promoting the growth of rice var Supanburi 1 and Pathumthani 80 in pot experiments in Chachengsao soil series*

Endophytic *C. brasiliense* resulted to promote the growth of rice var Supanburi 1 in Chashengsao soil. Nanomaterials derived from *Ch. brasilense* at 3 ppm treated for 15, 30 and 45 days showed significantly highest plant height of 17.85, 24.09 and 34.79 cm. which number of tillers were 2.08, 4.24 and 6.50, respectively when compared with the non-treated control (Table 5). It showed that crude extracts of *Ch. brasilense* at 100 ppm treated to rice for 15, 30 and 45 days showed significantly highest in plant height of 18.7, 27.76 and 38.63 cm. and the tiller numbers 2.41, 6.00 and

8.16, respectively (Table 5). Rice var Pathumthani 80 was treated with nanomaterials constructed from *Ch. brasiliense* at 3 ppm for 15, 30 and 45 days planted with Chashengsao soil showed significantly highest plant height of 15.72, 26.11 and 36.12 cm. and number of tillers were 4.04, 7.83 and 8.13, respectively (Table 6). Crude extracts from *Ch. brasilense* at 100 ppm for 15, 30 and 45 days resulted highest plant height of 17.60, 29.24 and 43.29 cm. and followed by number of tillers were 4.91, 9.00 and 9.53, respectively (Table 6).

***Endophytic Chaetomium brasilense for promoting the growth of rice var Supanburi 1 and Pathumthani 80 in pot experiment in Bangkok soil series***

Endophytic *Ch. brasilense* promoted the growth of rice var Supanburi 1 in Bangkok soil. Nanofibers derived from *Ch. brasilense* at 3 ppm for 15, 30 and 45 days resulted to be theb highest plant height of12.19, 19.92 and 24.44 cm which the number of tillers were 0.00, 0.25 and 0.58, respectively. Crude extracts from *Ch. brasilense* at 100 ppm for 15, 30 and 45 days showed the highest plant height of 13.47, 21.24 and 26.22 cm which the number of tillers of 0.00, 0.16 and 0.41, respectively. The endophytic *Ch. brasilense* proved to be promote plant growth of rice var Pathumthani 80 in Bangkok soil series. Nanofibers derived from *Ch. brasilense* at 3 ppm for 15, 30 and 45 days revealed the highest plant height of 15, 30 and 45 days which were 12.53, 19.36 and 23.97 cm which the number of tillers were not significantly differed at 15 days but varied at 30 and 45 days which were 1.66 and 5.16, respectively. Crude extracts from *Ch. brasilense* at 100 ppm for 15, 30 and 45 days showed plant height of 13.41, 21.30 and 26.74 cm which the number of tollers was not significantly differed at 15 days but significant difference at 30 and 45 days which were 3.33 and 7.33, respectively when compared the non-treated control.

**Table 5.** The growth of rice var Supanburi 1 in Chachengsao soil series

| **treatments** | **Plant height (cm)** | | | **Tiller number** | | |
|---|---|---|---|---|---|---|
| | **15 d** | **30 d** | **45 d** | **15 d** | **30 d** | **45 d** |
| T1 = control | 6.78$^{c1}$ | 14.90$^{d}$ | 24.42$^{d}$ | 0.00$^{b}$ | 0.00$^{c}$ | 1.91$^{c}$ |
| T2 = crude-CB | 18.7$^{a}$ | 27.76$^{a}$ | 38.63$^{a}$ | 2.41$^{a}$ | 6.00$^{a}$ | 8.16$^{a}$ |
| T3 = Nano-CB | 17.85$^{ab}$ | 24.09$^{b}$ | 34.79$^{b}$ | 2.08$^{a}$ | 4.24$^{b}$ | 6.50$^{ab}$ |
| T4 = spore suspensions-CB | 16.65$^{b}$ | 20.50$^{c}$ | 30.57$^{c}$ | 2.00$^{a}$ | 3.91$^{b}$ | 5.16$^{b}$ |
| C.V. (%) | 6.35 | 9.15 | 6.66 | 27.56 | 19.10 | 21.35 |

[1]Averages of four repeated experiments which followed by a common letter were not significantly differed by DMRT at P 0.01.

**Table 6.** The growth of rive var Pathumthani 80 in Chachengsao soil series

| treatments | Plant height (cm) | | | Number of tiller | | |
|---|---|---|---|---|---|---|
| | **15 d** | **30 d** | **45 d** | **15 d** | **30 d** | **45 d** |
| T1 = control | 11.65[d] | 19.45[d] | 28.04[d] | 0.50[b] | 3.16[c] | 3.33[c] |
| T2 = crude-CB | 17.60[a] | 29.24[a] | 43.29[a] | 4.91[a] | 9.00[a] | 9.53[a] |
| T3 = Nano-CB | 15.72[b] | 26.11[b] | 36.12[b] | 4.04[a] | 7.83[ab] | 8.13[ab] |
| T4 =spore suspensions-CB | 13.72[c] | 22.74[c] | 32.63[c] | 3.62[a] | 7.08[b] | 7.74[b] |
| C.V. (%) | 8.22 | 7.70 | 5.35 | 27.19 | 14.81 | 13.39 |

[1]Averages of four repeated experiments which followed by a common letter were not significantly differed by DMRT at P 0.01.

## Discussion

It is found that *Ch. brasilense* PT302, *Ch.cupreum* SP101 and *Ch.globosum* 01 were isolated from the healthy rice seedlings var Supanburi 1 and Prathumthani 80. Those isolates were morphology and molecular identified. *Ch. brasilense* PT302 was firstly selected for further experiment. Withthis, the former research finding found *Chaetomium* sp gave a good control of rice blast (Song *et al*., 2018). *Chaetomium* species were done using PCR technique at the position of ITS1-5.8S-ITS2 with primers ITS1 and ITS4 confirmed with DNA of 400-500 bp and compared with nucleatide sequences that related Chandra *et al.* (2015), Yew *et al.* (2014), Umamaheswari and Prabhakaran. (2012 ), Zakria Ahmed *et al*. (2016) and Ahammed *et al.* (2005). All isolates were confirmed as *Ch. brasilense*, *Ch.globosum* and *Ch. cupreum*. *Achaetomium strumarium* is used as out group (Rai *et al*. 1964; Abbott *et al*. 1995).

Endophytic *Ch. brasilense* PT302 showed plant growth promotion of rice var Supanburi 1 and Pathumthani 80 which similarly supported by Berjak and Villiers (1972), Basu and Pal (1979), Parera and Cantiffe (1994), Giri and Schillinger (2003), Carvalho *et al.* (2005) and Basra *et al.* (2006) who stated that endophytic microoorganism can be promoting plant growth. Crude extracts and nanofibers from endophytic *Ch. brasilense* resulted to increase seed germination of rice which similar to Syamsia *et al*. (2015) who stated that endophytes from rice promoted seed germination of rice in 7 days in Pulu Mandoti , Indonesia and its metabolite may be Indole acetic acid (IAA) and Gibberellic acid (GA) production, the rice plants were higher plant height and root than the non-treated ones (Waqas *et al*., 2012; Uthandi *et al.*, 2010). *Ch. globosum* is reported to produce IAA (Khan *et al*., 2011) and $GA_s$ (Rademacher, 1994; Bomke *et al*., 2008; Kawaide, 2006; Hamayun *et al*., 2010). The crude metabolite and nanofibers from *Ch. brasilense* is found to increase seed germination of rice which *Ch.*

*brasilense* is recorded to produce active compound chaetoglobosin C (Kanokmedhakul *et al.*, 2002). which these bioactive compounds could inhibit phytopathogens (Soytong *et al*., 2001; Sibounnavong *et al.*, 2012). Hyuncheol *et al.* (1998) found bioactive compound, Chaetochalasin A from *Ch. brasilense.* Morever, *Chaetomium* sp could be produced metabolites as IAA (Waqas *et al*., 2012 ; Uthandi *et al.*, 2010). Chaetoglobosin A from *Ch.brasilense* reported to inhibit plant pathogens (Hyuncheol *et al.,* 1988). These reports are in consistent with the result that crude metabolite and nanofibers from *Ch. brasilense* promoted the growth of rice var Supanburi 1 and Prathumthani 80.

Nanofibers and crude extracts from endophytic *Ch. brasilense* were sprayed to rice seedings var Supanburi 1 and Prathumthani 80 planted in Chachengsao and Bangkok soil series revealed that plant height and number of tillers had significantly higher than the non-treated control. Tongon and Soytong (2016) noted that fungal metabolites from *Ch. brasilense* inhibited the spore production of *Fusarium solani* causing tomato wilt and Sibounnavong *et al*. (2012) reported that crude hexane, crude EtOAc and crude MeOH from *Ch. brasilense* inhibited *F. oxysporum* f.sp. *lyc opersici* NKSC02 causing tomato wilt. Reddy *et al*. (1989) reported that endophytic *Ch. brasilense* released active metabolites and produce IAA to promote plant growth (Reddy *et al*., 1989). The fungal endophytes are reported to help in dissolving some plant nutrients eg. phosphate, zinc, potassium to be soluble forms for plant uptake (Nath. *et al*., 2015). The research findings stated that nanofibers derived from endophytic *Ch. brasilense* in rice plant gave significantly to promote plant growth at low concentration than crude metabolites. The nanofibers is promisingly rapid absorbed by plant cells.

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