
A new mycofungicide from *Emericella nidulans* against tomato wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* in vivo

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As a result, powder and oil formulations of *E. nidulans* gave highly significant at P=0.01 to control Fusarium wilt of tomato caused by *F. oxysporum* f.sp. *lycopersici* at 50 days after inoculated tomato seedlings with pathogen, the disease index were 1.75 and 2.00 where the wilt disease was decreased 63 % and 57 %, respectively. The followed effective treatments were treated with filtrate of *E. nidulans* and Procoraz (disease index were 2.5 and 3.5), the wilt disease was decreased 47% and 36 %, respectively when compared to the treatments of non-treated control (disease index was 1.00) and inoculated with pathogen. It is indicated that applying oil formulation of *E. nidulans* at the rate of 10 ml/20 L of water gave the best control tomato wilt caused by *F. oxysporum* f.sp. *lycopersici*. The disease was less and tomato plant grew better than the other treatments. Tomato treated with oil form of *E. nidulans* gave significantly highest in term of plant height than tomato treated with powder form of *E. nidulans*, filtrate of *E. nidulans* and procoraz chemical fungicide which non-significantly different when compared to the non-treated with pathogen (control) Fusarium wilt of tomato. In this study, the powder or oil formulation of *E. nidulans* have been made from spores including antagonistic substances or using only filtrate of *E. nidulans* gave effective control of tomato wilt. It is also noted that tomatoes treated with oil or powder forms of *E. nidulans* gave the highest root weight and followed by treated with filtrate of *E. nidulans*, procoraz and non-treated tomato with pathogen (12.50 g). It revealed that tomato treated with oil or powder forms of *E. nidulans* gave significantly highest total fruit weight per plant which were 582 and 533 g/plant and followed by tomatoes treated with filtrate of *E. nidulans*

Key words: *Emericella nidulans*, *F. oxysporum* f.sp. *lycopersici*, mycofungicide, tomato

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Introduction

Tomatoes (*Lycopersicon esculentum* Mill.) is the one of the most cultivated, popular and important vegetable crops in the world. Tomato wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* which is a fungus that become one of a limiting factor in the production of tomato and accounts for yield losses annually. It has become one of the most prevalent and damaging diseases wherever tomatoes are grown intensively because the pathogen persists indefinitely in infested soils. The methods used to control vascular wilt are either not very efficient or are difficult to apply. The best recommended way to control the disease is selecting resistant varieties of tomato (Silva and Bettiol, 2005). The pathogen has increased in the infested soil and become resistant to chemical fungicides. For this reason, alternative methods with emphasis on biological control using fungi or bacteria of controlling the disease have been studied by several researchers to reduce fungicide application and decrease cost of plant production. Recently, there have been many reports stated that some of fungi become a necessary to make a fungicide to control the diseases (Silva and Bettol, 2005; Soyong, 1992). Biological control of plant pathogens have been increasingly interested for plant pathologists and many researchers. It can serve as long-term protection of the disease as well as maintain environmental conditions for natural balance (Soyong, 1989). Recently there have been many reports about using bioactive compounds from biological control agents which were extracted from different fungi that inhibit many plant pathogenic fungi, including Fusarium wilt of tomato (Soyong, 1992). These bioactive compounds are Tricotoxin A50 extracted from *Trichoderma harzianum* PC01; and Chaetoglobosin C extracted from *Chaetomium globosum* that have been reported to elicit resistance or immunity in plants by inducing oxidative burst in plant cells (Nuchdonrong, *et al.*, 2004; Soyong, *et al.*, 2001).

The search for promising microbial antagonists have been increasingly interested and used to control the diseases. There are numerous reports indicated that *Chaetomium globosum* could control seedling blight of wheat caused by *Helminthosporium victoriae* (Tveit and Moore, 1954). Spraying the spore suspension of *Ch. globosum* could significantly control apple scab caused by *Venturia inaequalis* (Cullen *et al.*, 1984). *Chaetomium cupreum* is also reported to be antagonistic to *Phomopsis sojae* which caused seed-borne pathogen of soybean (Manandhar, 1986) and could significantly reduced the growth of seed-borne pathogen of rice e.g. *Curvularia lunata*, *Drechslera oryzae*, *Fusarium moniliforme* and *Pyricularia oryzae* (Soyong, 1992). *Ch. globosum* is reported to be significantly suppressed tomato wilt in Thailand caused by *Fusarium oxysporum* f.sp. *lycopersici* and *Pseudomonas*

solanaceurum (Soytong, 1990; 1991). It was also reported that *Ch. cupreum* could be controlled tomato wilt in the fields (Soytong, 1992). The effective strains of *Chaetomium* spp. have been formulated as biological products in the forms of pellet and powder (Soytong, 1993) and effectively controlled many soil borne plant pathogens (Soytong, 1996). Moreover, Phonkerd *et al.* (2008) reported that *Ch. cochlioides* strain Vth01 and CTh 05 can produce new dimeric spiro-azaphilones, cochliodones, two new azaphilones, chetoviridines E and F, a new epi-chaetoviridin A that exhibited antimalarial activity against *Plasmodium falciparum* and antituberculosis against *Mycobacterium tuberculosis*, and cytotoxicity against KB, BC1 and NCI-H187 cell lines. Moosophon *et al.* (2009) reported that chromatographic separation of the crude hexane extract from *Emericella rugulosa* strain ER could isolate new compounds of shamixanthone, isoemicellin, tajixanthone, tajixanthone methanoate, 14-methoxytajixanthone-25-acetate, and tajixanthone hydrate (Fig. 1). In this research finding of *E. nidulans* strain EN was firstly reported to release some antibiotic substances against human and plant pathogens. Some of these compounds exhibited activity towards *P. falciparum* (cause of malaria), *M. tuberculosis* (TB), *Candida albicans* and cancer cell lines (KB, BC and NCI-H187). The bioactivity evaluation of the isolated compounds are in progress and expressed to be inhibited some fungal plant pathogens. Those compounds also showed activity against plant diseases such as *Phytophthora* sp. causing root rot of plants and *Colletotrichum gloeosporioides* causing anthracnose disease (unpublished data).

In this study, it is revealed that the same isolate of *E. nidulans* strain EN could inhibit *F. oxysporum* f.sp. *lycopersici* caused tomato wilt could possibly imply antibiosis. Those bioactive compounds could be further tested to Fusarium pathogen as report of Sibounnavong *et al.* (2008) stated that could extracts of *E. nidulans* strain EN gave a good inhibition of *F. oxysporum* f.sp. *lycopersici*. The objective of this research was to develop and evaluate a new mycofungicide formulated from *E. nidulans* to control Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* *in-vivo*.

Materials and methods

Source of F. oxysporum and E. nidulans

Pure cultures of *F. oxysporum* f.sp. *lycopersici* were acquired from laboratory of Biocontrol Research Unit and Mycology Section, Department of Plant Pest Management, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Thailand. The cultures were transferred into Potato Dextrose Agar (PDA) and incubated at room temperature. The

morphological characteristic of *Fusarium* was studied under compound microscope. Pathogenicity test was done by following Koch's postulation method. Five tomato seedlings with root tips cut were soaked in different

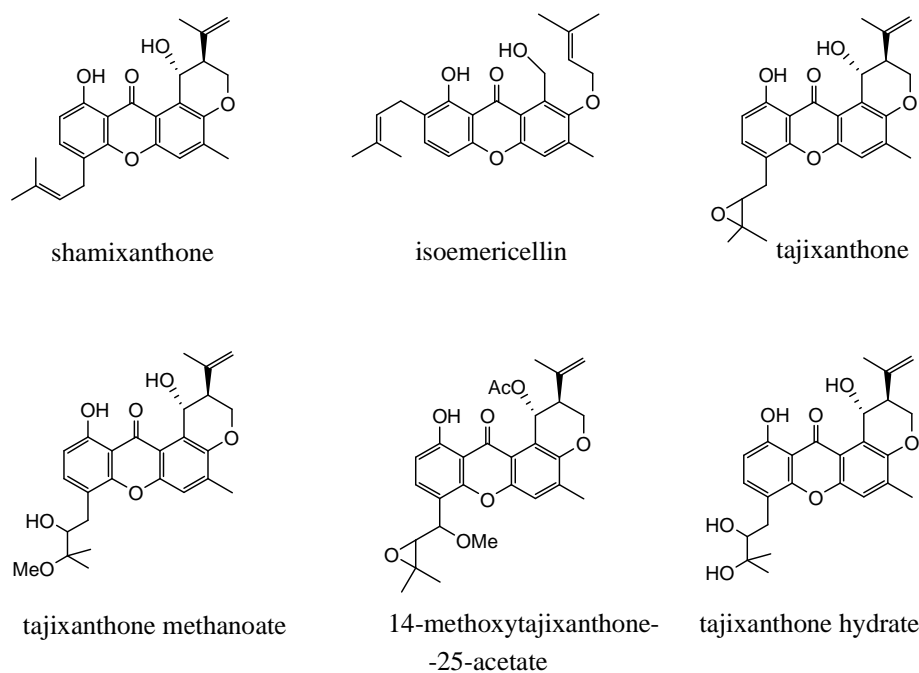


Fig. 1. Chemical structures of the isolated compounds from *Emericella rugulosa* strain ER (source: Moosophon *et al.*,2009).

strains with 1×10^8 spore/ml for 15 minutes before planting. The seedlings were planted in sterilized soils and the plants were rated for disease once the symptoms appear. The more pathogenic *Fusarium* strain was used in the study.

The promising antagonistic fungus, *Emericella nidulans* strain EN was acquired from Dr. Kasem Soyong. This was transferred onto PDA plates, and incubated at room temperature. The morphological characteristic of fungus was studied under compound microscope.

***In-vivo* assay of control tomato wilt in the pot experiment**

The potential of *E. nidulans*-based biofungicide was assessed *in-vivo* in pots of tomatoes. The formulated fungicides to be used are powder and suspension concentrations developed by Dr. Kasem Soyong and the culture

filtrate. Fifteen day-old tomato plants were inoculated with conidial suspension of *F. oxysporum* f. sp. *lycopersici* at concentration 1×10^6 conidia/ml from 7 day-old which culture on PDA by dipped root technique. The inoculated seedlings were grown in sterilized soil, which autoclaved at 121°C for 1 hour. Each treatment was performed which interval spray at every 10 days until harvest. Control was treated with sterile distilled water. The experiment was conducted using Randomized Completely Block Design (RCBD) with four replications. Each treatment consisted of three seedlings per replication. The following treatments were evaluated: Treatment 1 = Control, non-inoculated with pathogen and non-treated control, Treatment 2 = Control inoculated with pathogen and non-treated control, Treatment 3 = treated with powder formulation of *E. nidulans* at the rate of 10g/20 L of water. Treatment 4 = treated with oil formulation of *E. nidulans* at the rate of 10 ml/20 L of water. Treatment 5 = treated with culture filtrate of *Emericella nidulans*. Culture filtrate preparation:- *E. nidulans* was cultured in potato dextrose broth on electrical checker for 15 days, then removed mycelial mat through Whatman Filter Paper No. 4 to get the filtrate. The filtrate was then kept in refrigerator until use. Treatment 6 = chemical treated with Prochloraz as a common name of chemical fungicide at recommendation rate. Each treatment was performed which interval spray at every 10 days until harvest. Control was treated with sterile distilled water. The data were collected as plant height (cm), plant weight (g), number of fruits and weight of fresh ripen fruits (g). The diseased plants manifesting tomato wilt was longitudinally dissected and the infected vascular tissues were documented. Disease incidence was observed and rated at 30 days after inoculation based on a disease rating scale. Disease Index (DI) was scored as follows: 1= no symptom, 2= symptom on leaves 1-20%, lower leaves yellow, 3= symptom on leaves 21-40%, plants show yellowing or wilting of two leaves; 4= symptom on leaves 41-60%, plants show yellowing/wilting of two or more leaves, 5= symptom on leaves 61-80%, plants show vessel browning nearly to the leader shoot, with the most leaves wilt except the leader shoot, 6= symptom on leaves 81-100%, plants show wilt of leaves up to the shoot or died (Fig. 2).



Fig. 2. Disease Index (DI) was scored as follows: 1= no symptom, 2= symptom on leaves 1-20%, lower leaves yellow, 3= symptom on leaves 21-40%, plants show yellowing or wilting of two leaves; 4= symptom on leaves 41-60%, plants show yellowing/wilting of two or more leaves, 5= symptom on leaves 61-80%, plants show vessel browning nearly to the leader shoot, with the most leaves wilt except the leader shoot, 6= symptom on leaves 81-100%, plants show wilt of leaves up to the shoot or died. Statistical Analysis: Data were analyzed for significant differences using analysis of variance (ANOVA) and comparison among using Least Square Difference (LSD) and Duncan Multiple Range Test. SIRICHAH STATISTICAL PROGRAM 6 was used to analyze the data. The level of probability was set at P=0.05 and P=0.01.

Results and discussion

Source of *F. oxysporum* and *E. nidulans*

Fusarium oxysporum f.sp. *lycopersici* used in this study was confirmed pathogenicity as a virulent isolate.

In vivo assay to control tomato wilt in the pot experiment

Sterilized soil planted to tomatoes in pot was analysed by Department of Soil Science, Faculty of Agricultural Technology, King Mongkut's Institute of Technology (KMUTL), Ladkrabang, Bangkok, Thailand. It reported that soil type as loamy clay, pH 7.50, EC 835 us/cm, organic matter 3.32 %, available phosphorus (P) 228 ppm, potassium (K) 494 ppm, calcium (Ca) 2494 ppm, magnesium (Mg) 418 ppm, iron (Fe) 7.57 ppm, manganese (Mn) 13.3 ppm and

zinc (Zn) 6.82 ppm. It was predicted that the soil was nearly neutral status, high organic matter, high P, Ca, Mg and Fe but moderate level was Mn and Zn.

Results showed that *E. nidulans*-based biofungicide as powder formulation, oil formulation and culture filtrate were assessed in-vivo in pots of fifteen day-old tomato plants which were inoculated with conidial suspension of *F. oxysporum* f.sp. *lycopersici* at concentration 1×10^6 conidia/ml by dipped root technique. The inoculated seedlings were grown in sterilized soil. Each treatment was performed which interval spray at every 10 days until harvest. Control was treated with sterile distilled water.

As a result, powder and oil formulations of *E. nidulans* gave highly significant at $P=0.01$ to control Fusarium wilt of tomato caused by *F. oxysporum* f.sp. *lycopersici* at 50 days after inoculated tomato seedlings with pathogen, the disease index were 1.75 and 2.00 where the wilt disease was decreased 63% and 57%, respectively. The followed effective treatments were treated with filtrate of *E. nidulans* and Procoraz (disease index were 2.5 and 3.5), the wilt disease was decreased 47% and 36 %, respectively (Table 1 when compared to the treatments of non-treated control (disease index was 1.00) and inoculated with pathogen (disease index 4.75). Soyong (1992) reported that applying culture extract and Chaetomium formulation gave same level of significance to control Fusarium wilt of tomato.

In this study, the power or oil formulation of *E. nidulans* have been made from spores including antagonistic substances or using only filtrate of *E. nidulans* gave effective control of tomato wilt. This expression of antagonistic control, implied antibiosis that control mechanism may involve in antibiotic production of this fungus. The findings of Moosophon *et al.*(2009) reported that chemical constituents from *Emericella* sp. have been reported to be the source of a variety of natural products, mainly sesterterpenes with unusual polycyclic skeletons, for example, astellator, variecolin and prenylated xanthenes. The other species, *Emericella nidulans* were reported to produce the six compounds. Their structures were identified by spectroscopic methods as, epishamixanthone, shamixanthone, emericellin, ergosta-6, 22-diene-3-ol-5, 8-epidioxy-(3 β - 5 α , 22 E), sterigmatocystin and demethylsterigmatocystin.

Then, this research finding of applying *E. nidulans*-based biofungicide demonstrated successfully controlled Fusarium wilt of tomato that may possible be concerned on the secondary metabolites released during the fungal antagonistic growth to suppress the pathogen in nature. Udagawa *et al.* (1979) and Sekita *et al.* (1981) also stated that antibiotic substance in the form of mycotoxin was extracted and characterized from some *Chaetomium* spp. that could be inhibited some pathogen. This apparent success of biological control in these tests is due to the potent of *E. nidulans* which selected from natural

soil in a root zone of tomato plants may possible be applied in the field. The diseased plants manifesting tomato wilt was longitudinally dissected and the infected vascular tissues were documented. It is observed that the vascular bundle of infected tomato plant showed dark lines in both sides indicated that it was destroyed by the pathogen when compared to the healthy one as seen in Fig. 3. With this, Agrios (1997) stated that when the healthy plants grow in infested soil, the germ tube of spores or the mycelium penetrates root tips directly or enters the roots through wounds or at the point of formation of lateral roots. The mycelium advances through the root cortex intercellularly, and when it reaches the xylem vessels it enters them through the pits. The mycelium then remains in the vessels and travels through them, mostly upward, toward the system and crown of the plant. While in the vessels, the mycelium branches and produces microconidia. Finally, the vessel clogging by mycelium, spores, gels, gums and tyloses and crushing of vessels by proliferating adjacent parenchyma cells, is responsible for the breakdown of the water of the infected plant. When the leaves transpire more water than the roots and stem can transport to them, the stomata close and the leaves wilt and finally die, followed in death by the rest of the plant. In this study, the infected plant, most of which shows brown discoloration in vessels.

Plant height and fresh weight of stem and roots were shown in Table 2. It is indicated that applying oil formulation of *E. nidulans* at the rate of 10 ml/20 L of water gave the best control tomato wilt caused by *F. oxysporum* f.sp. *lycopersici*. The disease was less and tomato plant grew better than the other treatments. Tomato treated with oil form of *E. nidulans* gave significantly highest in term of plant height (165.50 cm) than tomato treated with powder form of *E. nidulans* (94.50 cm), filtrate of *E. nidulans* (88.00 cm) and procoraz chemical fungicide (74.50 cm) which non-significantly different when compared to the non-treated with pathogen (95.00 cm). However, all treated tomatoes and non-treated tomato with pathogen (control) were significantly higher plant height than the inoculated tomatoes (65.50 cm). It is also noted that tomatoes treated with oil or powder forms of *E. nidulans* gave the highest root weight (16.25 g) and followed by treated with filtrate of *E. nidulans* (16.00 g), procoraz (12.50 g) and non-treated tomato with pathogen (12.50 g). All treated tomatoes and non- treated tomato with pathogen gave significantly higher root weight than tomatoes inoculated with pathogen (8.35 g). It is revealed that all treated tomatoes and non-treated tomato with pathogen (control) were significantly higher plant fresh weight than the inoculated one (Fig. 4). With this, Soyong (1990) state that *Chaetomium* mycofungicide applied to inoculate tomato to control *F. oxysporum* f.sp. *lycopersici* also expressed higher plant growth parameters than the non-treated one.

It revealed that tomato treated with oil or powder forms of *E. nidulans* gave significantly highest total fruit weight per plant which were 582 and 533 g/plant and followed by tomatoes treated with filtrate of *E. nidulans* (430 g/plant). All tomatoes treated and non-treated tomato with pathogen (control) were significantly higher total fruit weight than the inoculated tomatoes. The number of fruit per plant was also significantly highest in tomato treated with oil form of *E. nidulans* (43.75 fruit/plant) and followed by tomatoes treated with powder form of *E. nidulans* and filtrate of *E. nidulans* which were 28.25 and 25 fruits per plant. The other treatments including prochoraz and non-treated tomato with pathogen (control) were significantly higher number of fruit per plant than the inoculated tomatoes (Table 3). It is observed that in this experiment. This may possible due to the pathogen resist to the chemical fungicide as stated by (Silva and Bettiol, 2005; Soyong, 1992).

Table 1. Disease index of tomato wilt.

Treatments	Disease index (DI)			
	25 days	45 days	60 days	DD(%) ²
Non-treated with pathogen	1.00b ¹	1.00b	1.00d	-
Inoculated with pathogen	1.75b	2.25a	4.75a	-
Powder form of <i>E. nidulans</i>	1.00b	1.25ab	1.75cd	63.15
Oil form of <i>E. nidulans</i>	1.00b	1.50ab	2.00cd	57.89
Filtrate of <i>E. nidulans</i>	1.00b	1.50ab	2.50bc	47.36
Prochoraz	1.00b	2.25a	3.50b	36.84

¹Average of four replications. Means follow by a common letter in each column are not significantly difference by DMRT at P = 0.01.

²Percentage of Decreased Disease (% DD) = DI of inoculated with pathogen – DI of treated one/DI of inoculated with pathogen X 100.

Table 2. Plant height and fresh weight of tomatoes at 80 days.

Treatments	Plant height (cm)	Plant fresh weight	
		stem (g)	roots(g)
Non-treated with pathogen	95.00ab ¹	146.25a	15.25ab
Inoculated with pathogen	65.50b	63.00b	9.00b
Powder form of <i>E. nidulans</i>	94.50ab	150.00a	16.00ab
Oil form of <i>E. nidulans</i>	105.50a	160.00a	19.50a
Filtrate of <i>E. nidulans</i>	88.00ab	142.00a	12.50bc
Prochoraz	74.50ab	123.75a	14.00ab

¹ Average of four replications. Means follow by a common letter in each column are not significantly difference by DMRT at P = 0.01.

Table 3. Total fruit number and weight per plant.

Treatments	Number (fruit)	Weight/plant (g)
Non-treated with pathogen	19.25bc ¹	275.00c
Inoculated with pathogen	11.00c	145.00e
Powder form of <i>E. nidulans</i>	28.25b	533.75a
Oil form of <i>E. nidulans</i>	43.75a	582.50a
Filtrate of <i>E. nidulans</i>	25.00b	430.00b
Prochloraz	16.00bc	200.00d

¹Average of four replications. Means follow by a common letter in each column are not significantly difference by DMRT at P = 0.01.

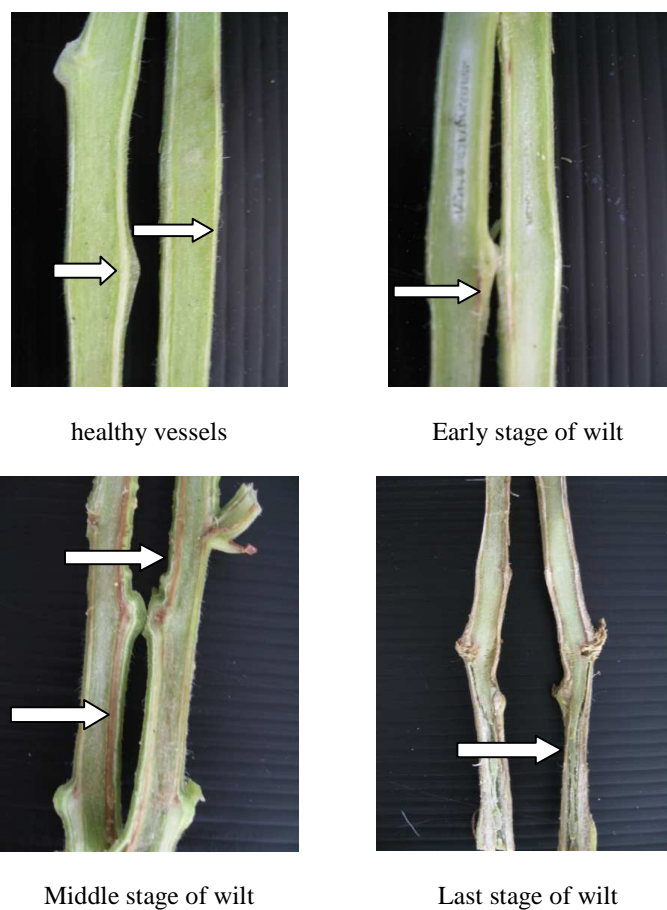


Fig. 3. The diseased plants manifesting tomato wilt was longitudinally dissected and the infected vascular tissues caused by *Fusarium oxysporum* f.sp. *lycopersici*.

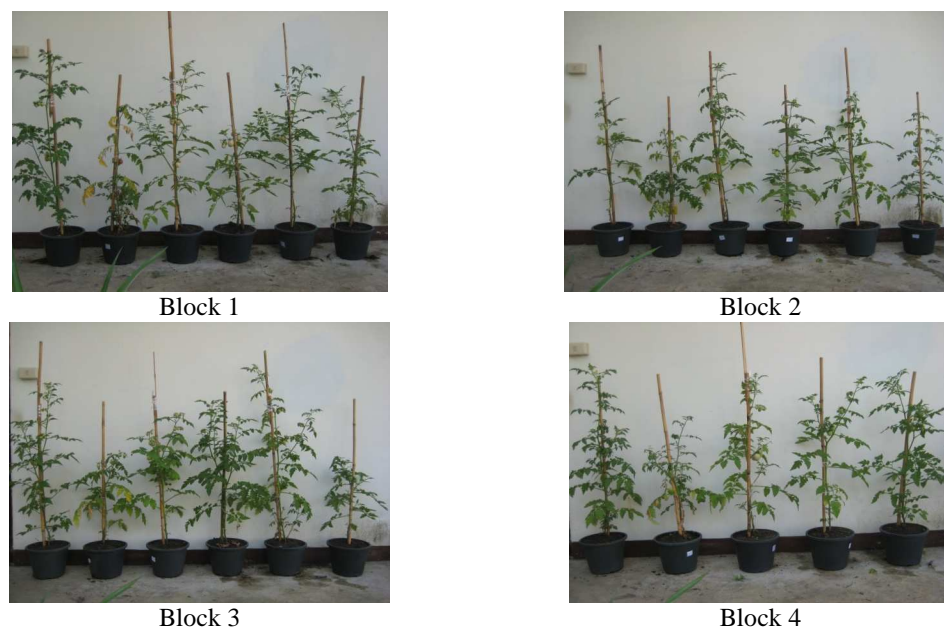


Fig. 4. Testing of biological fungicide formulated from *E. nidulans* with different formulation compared to chemical fungicide to control tomato wilt caused by *F. oxysporum* f.sp. *lycopersici*. From left to right:- T1 = Control, non-inoculated with pathogen and non-treated control, T2 = Control inoculated with pathogen and non-treated control, T3 = treated with powder formulation of biofungicide at the rate of 10 g/20 L of water, T4 = treated with oil formulation of biofungicide at the rate of 10 ml/20 L of water, T5 = treated with culture filtrate of *E. nidulans* and T6 = chemical treated with Prochloraz as chemical fungicide at recommendation rate.

References

- Agrios, G.N. (1997). Plant Pathology. 4th edition. Academic Press. San Diego, California. USA. 635 p.
- Cullen, D., Berbeem, F.M., and Andrews, J.H. (1984). *Chaetomium globosum* antagonizes the apple scab pathogen, *Venturia inaequalis*, under field conditions. *Can. J. Bot.* 62: 1814-1818.
- Khumkomkat, P., Kanokmedhakul, S., Kanokmedhakul, K., Hahnvajanawong, C. and Soyong, K. (2009). Antimalarial and cytotoxic depsidones from the fungus *Chaetomium brasiliense*. *J. Nat. Prod.* 72: 1487-1491.
- Manandhar, P.N., Thapliyal, P.N. and Sinclair, J.B. (1986). Potential biocontrol fungi for selected soybean fungal pathogen. *Biological Control and Cultural Test* 1: 36.
- Moosophon, P., Kanokmedhakul, S., Kanokmedhakul, K. and Soyong, K. (2009). Prenylxanthenes and a bicyclic (3.3.1) nona-2,6-diene derive from the fungus *Emericella rugulaosa*. *J. Nat. Prod.* 72: 1442-1446.

- Nuchadomrong, S., Budthongsri, P., Daduanf, S., Kanokmedhakul, S., Soytung, K. (2004). *Trichotoxin A50*: Induction of an oxidative defense mechanism in tomato and its cellular site of action. In Proceeding 30th Congress on Science and Technology of Thailand, 19-21 October, Impact Muang Thong Thani, Bangkok, Thailand.
- Phokerd, N., Kanokmedhakul, M. S., Kanokmedhakul, K., Soytung, K., Prabpai, S. and Kongsearee, P. (2008). Bis-spiro-azaphilones and azaphilones from the fungi *Chaetomium cochliodes* VTh01 and *C. cochliodes* CTh05. *Tetrahedron* 64: 9636-9645.
- Sekita, K., Yoshihira, S., Natari, S., Udagawa, S., Muroi, T., Sugiyama, H., Kurata, H. and Umeda, M. (1981). Mycotoxin production by *Chaetomium* spp. and related fungi. *Can. J. Bot.* 27: 766-772.
- Silva, J.C. and Bettol, W. (2005). Potential of non-pathogenic *Fusarium oxysporum* isolate for control of fusarium wilt of tomato. *Fitopatologia Brasileira* 30: 409-412.
- Sibounnavong, P., Cynthia, D.C., Kanokmedhakul, S. and K. Soytung. 2008. The new antagonistic fungus, *Emericella nidulans* strain EN against Fusarium wilt of Tomato. *Journal of Agricultural Technology* 4(1): 89-99.
- Sibounnavong, P., Soytung, K., Divina, C.C., and Kalaw, S. (2009). In – vitro biological activities of *Emericella nidulans*, a new fungal antagonist, against *Fusarium oxysporum* f. sp. *lycopersici*. *Journal of Agricultural Technology* 5(1): 75-84.
- Soytung, K. (1990). Biological control of tomato wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* using a new biofungicide. A paper presented at the International Conference on Biotechnology and Environmental Science, August, 21-24, 1990. Chulabhorn Research Institute (Abstract).
- Soytung, K. (1991). Species of *Chaetomium* in Thailand and screening for their biocontrol properties against plant pathogens. A paper presented at the XII International Plant Protection Congress. Rio de Janeiro, Brazil, 11-16 August, 1991. (abstract), supported by IFS, Sweden.
- Soytung, K. (1992). Biological control of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* using *Chaetomium cupreum*. *Kasetsart J. (Nat. Sci.)* 26: 310-313. And International Conference on Biological Control in Tropical Agriculture. Malaysia. August 27-30, 1991. p. 135 (IFS Travel Grant).
- Soytung, K. (1993). Encapsulation of potential biocontrol agents in sodium alginate. The 6th International Congress of Plant Pathology. Canada. July, 28-August, 6, 1993. P. 273.
- Soytung, K. (1996). *Chaetomium* as a new broad spectrum mycofungicide. Proc. of the first International Symposium on Biopesticides, October 27-31, 1996, Thailand. 124-132 pp
- Soytung, K. and Quimio, T.H. (1989). A taxonomic study on the Philippine species of *Chaetomium*. *The Philippine Agriculturist* 72(1): 59-72.
- Soytung, K., Kanokmedhakul, S., Kukongviriyapa, V. and 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.
- Tveit, M. and Moore, M.B. (1954). Isolates of *Chaetomium* that protect oats from *Helminthosporium victoriae*. *Phytopathology* 44: 686-689.
- Udagawa, S., Muroi, T., Kurata, H., Sekita, S., Yoshihira, S. and Natori, S. (1979). *Chaetomium udagawae*: A new producer of sterigmatocystin. *Trans. Mycol. Soc. Japan.* 20: 475-480.

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