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Screening of *Emericella nidulans* for biological control of tomato *Fusarium* wilt in Lao PDR

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The isolate VTS16 was significantly highest disease index of tomato wilt caused by *F. oxysporum f sp lycopersici* var Sida which categorized as high virulent. *E. nidulans* isolate L01 is screened to be the most potential antagonistic fungus against *F. oxysporum* f sp *lycopersici* which inhibited spore production of 82.05 %. Crude methanol of *E. nidulans* isolate L01 expressed antifungal activity against *F. oxysporum* f sp *lycopersici* at the ED_{50} of 112 µg/ml, and follwed by crude ethyl acetate and crude hexane which were 379 and 915 µg/ml, respectively. Thereafter, *E. nidulans* L01 cultured on PDB at pH 8 and mixed PDB and CWDB at pH6 produced the highest fungal biomass and suitable to propagate for spore production.
Disease index in oil based formulation produced from *E. nidulans* isolate L01 gave the lowest wilt incidence of tomato var Sida (DSI 1.75) and followed by powder based formulation (DSI 2.00), culture filtrate (DSI 2.75) and prochoraz (DSI 3.50) when compared to inoculated control (DSI 4.75). It is shown that oil based formulation showed significantly better plant height (119.25 cm) than powder based formulation which plant height was 109.75 cm and followed by culture filtrate and prochoraz which plant height were 84.50 and 73.00 cm, respectively when compared to the inoculated control (62.75 cm). Powder and oil based formulations gave the plant weight of 91.75 and 98.50 g/plant which better in plant weight than culture filtrate and prochoraz (68.00 and 67.00 g/plant) which non-significantly differed when compared the inoculated control (64.00 g/plant). Results in root weight and fruit number/plant were similar to those in plant weight. Tomato treated with oil and powder based formulation of *E. nidulans* isolate L01 gave the highest yields (fruit weight) of 218.50 and 197.50 g/plant, respectively and followed by treated with culture filtrate and prochoraz which yielded 128.00 and 107.00 g/plant, respectively when compared to the inoculated control (83.75 g/plant). As a result, it is indicated that power and oil based formulations increased in plant growth parameters 30-60 % when compared to inoculated control. Oil and powder based formulations reduced the wilt incidence of 63.15 and 57.89 % and followed by culture filtrate and prochoraz which reduced wilt incidence of 42.10 and 36.31 %, respectively.

Key words: *Emericella nidulans, Fusarium oxysporum* f. sp. *lycopersici*, crude extracts, bio-agent formulations

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is widely cultivated as vegetable crops in the world. *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen causes wilt of tomato mostly in upland areas which cause economic losses. *F. oxysporum* f. sp. *lycopersici* causing tomato wilt has become difficult to control wherever tomatoes are intensively grown and persisted in the infested soils (Agrios, 1997). The disease controls are managed either inefficient or difficult by using the chemical fungicides. Over time tomatoes have been reported to develop resistant to some races of *F. oxysporum* f. sp. *lycopersici* or the pathogen may also develop resistant to chemical fungicides (Silva and Bettiol, 2005). The excessive and misuse of a wide range of fungicides has led to harmful to environment and increase resistant pathogen populations (Soytong *et al*., 2001).

The available control methods for Fusarium wilt of tomato are either inefficient or difficult to apply (Alabouvette *et al*., 1998). Biological control of plant disease using of beneficial microorganisms would help to reduce the chemical fungicide application and decrease cost of production. It has been proved the effective biological control agents through antibiosis, competition, suppression, direct parasitism and induced resistance. (Haggag and Mohamed, 2007 and Larkin and Fravel, 1998). Reports on biological control of Fusarium wilt have been increased using *Trichoderma harzianum*, *Pythium oligandrum*, *Achromobacter xylosoxydans*, *Penicillium oxalicum*, non-pathogenic *Fusarium oxysporum*, *Chaetomium globosum* and *Chaetomium cupreum* (Floch *et al*., 2003; De Cal *et al*., 2000; Moretti *et al*., 2008; Silva and Bettiol, 2005; Soytong *et al*., 2001). Moreover, Larkin and Fravel (1998) reported that non-pathogenic *Fusarium* spp. could reduce the incidence of tomato wilt between 50–100% while *Trichoderma* spp and *Gliocladium* spp. reduced wilt incidence between 37–75% and *Pseudomonas* spp. reduced wilt incidence of 30–63% in field trial. Suwan *et al*. (2000) mentioned that mycelial extracts of *Trichoderma harzianum* PC01 which produce Trichotoxin acted as a potent biological control agent. The extract could inhibit mycelial growth and sporangial production of *Phytophthora palmivora* with ED_{50} values of 2.2 and 0.45 mg/ml. The mechanism of antibiosis plays the important role of production of secondary metabolites against plant pathogens. Some specific isolates of *Trichoderma* spp have reported to produce volatile and non-volatile antifungal substances, such as 6-n-pentyl-6H-pyran-2-one (6PP), gliotoxin, viridin, harzianopyridone, harziandione and peptaibols. *Trichoderma* spp. was also reported to be enhanced plant defense responses to pathogen attack (Vinale *et al*., 2008). *C. globosum* produces cell wall hydrolases (such as chitinase and

glucanase) and antibiotics (such as chaetoglobosin and chaetomanone), which inhibit fungal plant pathogens (Kanokmedhakul *et al*., 2002; Park *et al*., 2005). These are involoved in biocontrol mechanism. Soytong *et al*. (2001) stated that Chaetoglobosin C, the bioactive compound extracted from *C. globosum*, act as alien substance which induced a localized and sub-systemic oxidative burst in tomato, tobacco, potato, and carrot. This possibility acts as an induction of plant immunity for disease resistance. Moreover, crude extract of *C. cupreum* inhibited spore production of *F. oxysporum* f. sp. *lycopersici* causing tomato wilt var Sida at 85.14% (Soytong, 1992).

Emericella spp. belongs to the Ascomycota, and have been reported as antagonistic fungi against *F. oxysporum* f. sp. *lycopersici* (Sibounnavong *et al*., 2010). *Emericella nidulans* and *Emericella rugulosa* has been reported to produce antibiotic substances, five prenylxanthones, ruguloxanthones A–C, 14-methoxytajixanthone,-tajixanthone ethanoate, a bicycle[3.3.1] –nona-2,6-diene derivative named rugulosone, shamixanthone, tajixanthone, 14-methoxytajixanthone-25-acetate, tajixanthone hydrate, tajixanthone methanoate, isoemericellin and ergosterol. Among these, the bicyclo[3.3.1]–nona-2,6-diene derivative exhibited not only antimalarial, antimycobacterial activity and cytotoxicity against three cancer cell lines (Moosophon *et al*., 2009) but also antifungal against Fusarium wilt pathogen (Sibounnavong *et al*., 2009). *E. nidulans* has been firsltly reported as a new biological control agent to antagonize *F. oxysporum* f. sp. *lycopersici* causing tomato wilt (Sibounnavong *et al*., 2010).

The objectives of research project were to isolate *F. oxysporum* f. sp. *lycopersici* causing tomato wilt and pathogenicity proved to screen the virulent isolate for pathogenicity. Isolation and screening of *Emericella* spp. as antagonistic fungi against *F. oxysporum* f. sp. *lycopersici* were done. Bi-culture antagonistic and crude extract tests against *F. oxysporum* f. sp. *lycopersici* were proved it control mechanism. The research finding for optimum growth of effective isolate *Emericella* spp. in different media and pH levels were investigated for mass production. Bio-agent formulations of effective isolate *Emericella* spp were performed and evaluated their efficacy. Testing bio-agent formulations of the effective *Emericella* spp. to control Fusarium wilt of tomato var. Sida in pot experiment was also evaluated.

Materials and methods

Pathogen and pathogenicity test

Fusarium oxysporum f. sp. *lycopersici* was isolated from tomato cultivation in Laos PDR by using tissue transplanting and soil plate method according to the work of Soytong (1992). All isolates were tested for pathogenicity using tomato seedling var. Sida by inoculating root-dipped with conidial suspension of pathogen $1x10^7$ conidia/ml. Disease severity index (DSI) was scored at 21 days after inoculation based on the modified disease severity of Sibounnavong *et al* (2010) as follows:- 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21-40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting 81-100% or die. The most virulent isolate was selected for further experiment. The test was done using Completely Randomized Design (CRD) with four replications and repeated twice. Data collection was computed analysis of variance and means were compared using Duncan's Multiple Range Test (DMRT) at P = 0.05 and 0.01.

Emericella nidulans as antagonistic fungus

Emericella spp. were isolated from forest soil in Lao PDR using soil plate technique on glucose asparagines nitrate agar. The isolates were cultured on poato dextrose agar and tested to screen its biological control potential to inhibit the most virulence isolate of *F. oxysporum* f. sp. *lycopersici*. The most effective antagonistic fungus of *E. nidulans* was sub-cultured on potato dextrose agar (PDA) for further study.

Bi-culture antagonistic test

The test was conducted by using the method of Soytong (1992) and Sibounnavong *et al*. (2008 and 2009a). The most promising antagonistic fungus *E. nidulans* and the virulent isolate of *F. oxysporum* f. sp. *lycopersici* were made bi-culture on PDA for 7 days, incubated at the room temperature (28-30ºC). The edge of active colony growth of pathogenic fungus and promising antagonistic fungus was taken with 0.5 mm diameter by the sterilized cork borer and one agar plug of each fungus was transferred to the opposite sides on the PDA plates of 9 cm diameter and separately culture of antagonistic fungi and pathogen served as a controls, incubated at the room temperature (28-30ºC) for three to four weeks. Data were collected as colony diameter (cm) and spore

production counted on Haemacytometer under binocular compound microscope. The experiment was done using CRD with four replications. Data collection were recorded as colony diameter (cm), number spore production of tested pathogen, and computed the analysis of variance (ANOV), then compared treatment means using Duncan's Multiple Range Test (DMRT) at P = 0.05 and 0.01. The experiment was repeated in two times.

Growth of Emericella nidulans culture in different media and pH levels

The most effective of *E. nidulans* was further studied on the optimum growth for bio-agent formulation. The experiment was conducted using two factor factorial experiment in Completely Randomized Design (CRD). Factor A - kinds of media, A1 = PDB (Potato Dextrose Broth); A2 = CWDB (Coconut Water Dextrose Broth); A3 = mixed between PDB and CWDB, Factor B – pH levels, B1 = 5, B2 = 6, B3 = 7 and B4 = 8. PDB was prepared by boiling 200g of potato in 1000 ml of water and mixed with 20g of dextrose. CWDB was prepared by boiling 1000 ml of coconut water and mixed with 20g of dextrose. The media were separated in 20 ml of medium in each flask and adjusted the pH levels by adding either HCl or NaOH to get the required pH levels, then sterilized by autoclaving at 121° C, 15 lbs/inch^2 for 20 min. An agar plug of *E. rugulosa* was transferred into the middle of medium in each Petri dish and incubated for 7 days at room temperature (approximately, 28-30 °C). After 7 days, the culture on each treatment was separately filtered using Whatman filter paper No 4 to get fresh fungal biomass. The fungal biomass was air dried at room temperature for 48 hours. Fresh and dried fungal biomass were weighted (mg) using electrical balance. The most suitable medium and pH level for the fungus growth was used as based medium to culture *E. nidulans* for preparing the fungal biomass to formula the biofungicide to control Fusarium wilt of tomato in the pot experiment.

Crude extract test against pathogen

It was also tested the mechanism of control in term of antibiosis of the most effective *E. nidulans*. The crude extracts from *E. nidulans* was performed followed the method of Kanokmedhakul *et al.* (2006), Moosophon *et al.* (2009), and Thohinung *et al*. (2010). *E. nidulans* was cultured in potato dextrose broth (PDB) at room temperature (30–32ºC) for 30 days. The dried fungal biomass was ground and sequentially extracted with hexane, ethyl acetate, and methanol. The solvents were then evaporated *in vacuo* to yield crude hexane, crude ethyl acetate (EtOAc), and crude methanol (MeOH)

extracts, respectively. The crude extracts were assayed for inhibition of the most virulent isolate of *F. oxysporum* f. sp. *lycopersici*. The experiment was conducted by using a factorial experiment in CRD with four replications. Factor A represented the different solvents: A1 = crude hexane, A2 = crude ethyl acetate and A3 = crude methanol. Factor B represented the different concentrations: B1 = 0 µg/ml (control), B2 = 50 µg/ml, B3 = 100 µg/ml, B4 = 500 µg/ml and B5 = 1,000 µg/ml. Each crude extract was dissolved in 2% dimethyl sulfoxide and added to PDA before autoclaving at 121°C (15 psi) for 30 minutes. To perform the assay, a sterilized 3-mm diameter cork borer was used to remove agar plugs from the actively growing edge of the pathogen culture. An agar plug was transferred to the center of 5 cm diameter Petri dishes of PDA containing crude extract at each concentration and incubated at room temperature (30–32ºC) until the pathogen on the control plates had grown over the plate. Data were collected regarding the number of conidia produced by the pathogen and used to calculate the percentage of conidia inhibition. The effective dose (ED_{50}) was calculated using Probit analysis. The experiment was repeated twice. The most effective crude extract was used for study on effect of fungal metabolites on disease incidence.

Testing bio-agent formulations to control Fusarium wilt of tomato

Bio-agent formulations were separately formulated as powder and oil based formulation according to the method of Soytong (2001) by using fungal biomass of *E. nidulans* which culture on the optimum pH and medium test from previously experiment. The culture of *E. nidulans* was incubated at room temperature (28-32˚C) for 30 days. The ascospores of *E. nidulans* was collected and adjusted to 2 x 10^7 spores/ml, then added into sterilized palm oil as to formulate the based oil bio-agent form or added to sterilized talcum as based powder bio-form. Bio-agent formulations of *E. nidulans* were evaluated for their abilities to control tomato wilt by inoculated *F. oxysporum* f. sp *lycopersici* to tomato seedlings var. Sida. Tomato seedlings at 30 days old were inoculated with conidial suspension of *F. oxysporum* f. sp. *lycopersici* at concentration of 1x10^7conidia/ml by dipping root for 15 min before transplanting into plastic pot contained with sterilized mix soil (soil:sand:compost, 4:1:1). Soil mixture was sterilized at 121 C, 15 lbs/inch^2 for 1 h in two consecutive days.

The experiment was conducted using Randomized Completely Block Design (RCBD) with four replications. Treatments were designed as follows:- non-inoculated control (T_1), inoculated with pathogen and non-treated bio-agent formulation (T_2), culture filtrate of *E. nidulans* (T_3), powder bio-agent

formulation (T4), oil bio-agent formulation (T_5) and chemical fungicide, prochoraz 50% WP (T_6). Each treatment was separately applied at the rate of 20 ml/ 20 L of water, while powder bio-agent formulation and prochoraz 50% WP were applied at the rate of 20 g/ 20 l of water at every 2 weeks by spraying around rhizosphere soil and above plants. Data were collected as disease severity index (DSI), plant fresh weight (g), and fruit weight or yield (g). Disease severity index (DSI) was scaled as previous experiment. Percentage of disease reduction was analyzed using formula: % disease reduction = (Disease severity index of control – Disease severity index of treatment)/ Disease severity index of control x 100. Plant fresh and dry weights (g) and fruit weight (g) were recorded at harvested day. Percent increased in yield was analyzed using formula: (yield per plant in treatment – yield per plant in control)/ yield per plant in treatment x 100. All data were subjected to analysis of variance (ANOVA). Treatment means were statistical compared with Duncan's New Multiple Range Test (DMRT) at $P \leq 0.05$ to separate means. The experiment was repeated two times.

Results

Pathogen and pathogenicity test

Twenty isolates of *F. oxysporum* was encountered and cultured on PDA for morphologically study under binocular compound microscope. In general, culture on PDA appears as fast-growing colony, reaching 9 cm diameter in 7-15 days at room temperature. Aerial mycelium sparse to abundant and becoming felted, whitish purple or vaiable in color in some isolates. Philophores are short and formed singly and branched. Macroconidia shape is fusiform, slightly curved, 3-5 septate, 13.0-34.0 x 2.5-3.5 µm. Microconidia abundant, 0-1 septate, elliptical or cylindrical, straight, 4.0 x 1.5 µm and chamydospores are in chain with either terminal and intercalary (Figure 1) as similar to Domsch and Gams (1993).

Pathogenicity showed that isolate VTS16 was significantly highest disease index of tomato wilt caused by *F. oxysporum f sp lycopersici* var Sida which categorized as high virulent and followed by isolate VTS17 which showed medium virulent. The majority of isolates showed low virulent group as follows:- VTS 01, 02, 03,04,05,06,07,08,09,10,11,12,13,14,15,18 and 19. Only isolate of VTS 20 was avirulence as shown in Table 1.

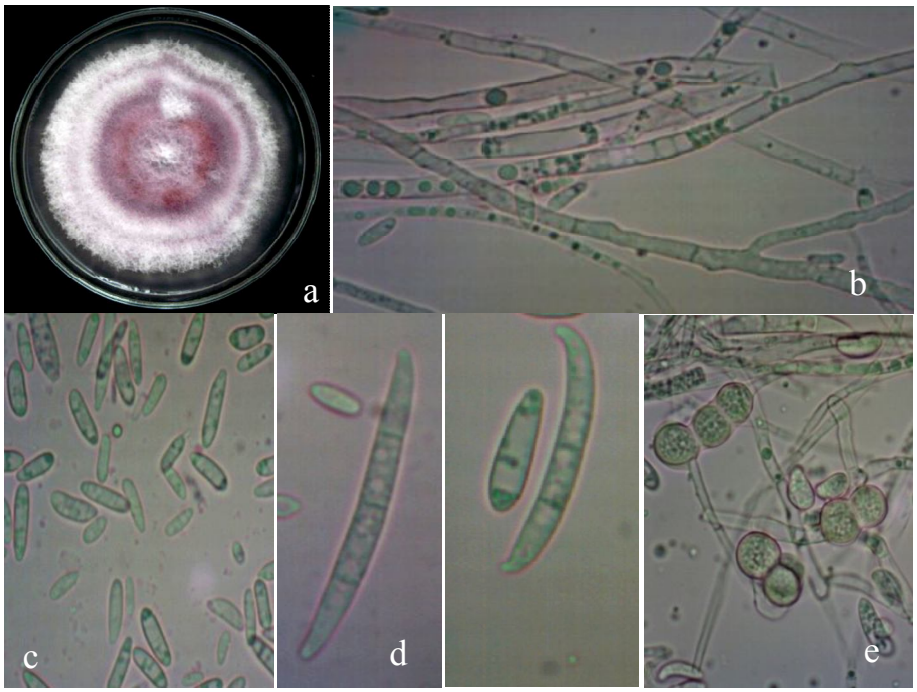


Fig. 1. Morphological character of *Fusarium oxysporum* f.sp. *lycopersici* at 15 days.
a. Colony on PDA, b. mycelium, c. microconidia, d. macroconidia, e. chlamydospores.

Emericella nidulans as antagonistic fungus

All isolates of *Emericella nidulans* were tested to screen it biiocontrol potential against *F. oxysporum* f sp *lycopersici*. Each isolate was cultured on PDA for identification work. It is observed that all encountered isolates appear imperfect or anamorphic stage namely *Aspergillus nidulans* (Eidam) Winter. Colony diameter on PDA averaged 5-6 cm in two weeks at room temperature (28-30ºC), green from conidia becoming brownish when mature to produce abundant Cleistothecia, dull yellow, globoseor oval sharp 122 x 345.0 µm in diameter, surrounded by hülle cells. Hülle cells ellipsoidal to globose, 9.0-20.0 µm in diameter. Asci are globose to subglobose, 8-spored. Ascospores are red to purple, smooth, 2.5-4.0 × 3.4-5.5 µm, with two narrow longitudinal furrows as similar described by Domsch and Gams (1993) as shown in Figure 2.

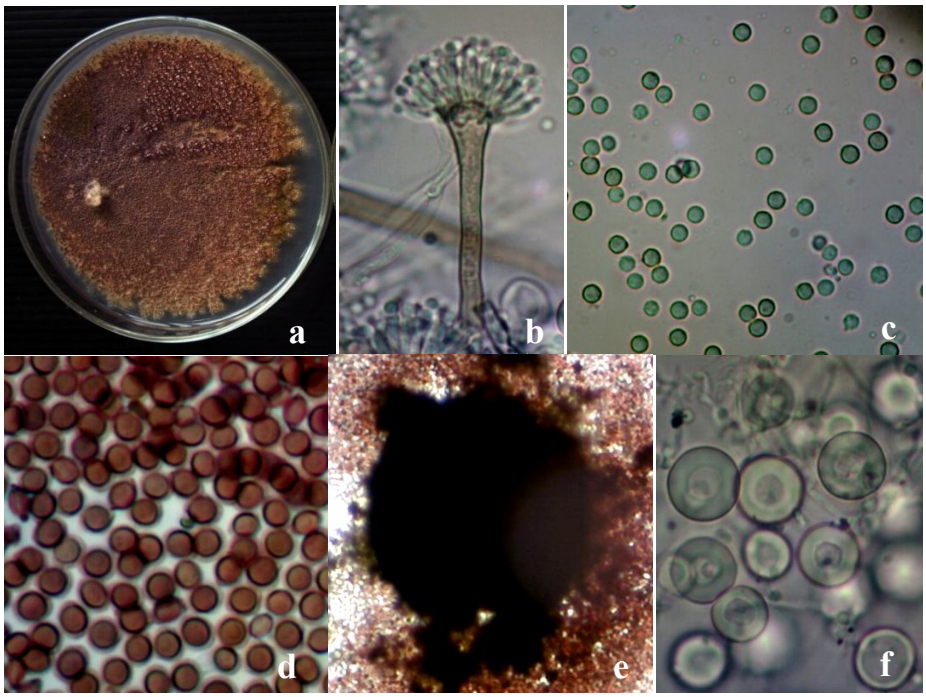


Fig. 2. Morphological character of *Emericella nidulans* on PDA at 15 days
a. Emericella nidulans on PDA, b. Thallus in imperfect stage, c. conidia, d. ascospores in perfect stage, e. Cleistothecium or ascomatum , f. hülle cells

Bi-culture antagonistic test

The colony diameter of *F. oxysporum f sp lycopersici* isolate VTS16 in bi-culture plate averaged 5.83 cm while in control plate was 9.00 cm. The conidial number of *F. oxysporum* f sp *lycopersici* VTS16 in bi-culture plate averaged 0.69 x 10^7 conidia/ml while in control plate was 2.70x10^7 conidia/ml. It showed that *E. nidulans* isolate L01 significantly inhibited colony growth of 35.25 % and inhibited spore production of 82.05 % as shown in Figure 3. The other isolates of *E. nidulans* that no potent to inhibit the conidial production were screened out.

Growth of E. nidulans culture in different media and pH levels

Results showed that *E. nidulans* isolate L01 which cultured in PDB at pH 8 and mixed media of PDB and CWDB at pH6 produced more fungal biomass which gave highly significant in fresh weight of fungal biomass than other treatments. However, CWDB medium at pH 5, 6, 7, and 8 was not suitable for

growth of *E. nidulans* which gave lowest of fungal biomass in the medium at pH levels of 5, 6, 7, and 8 respectively (Table 2). This result suggested that the *E. nidulans* isolate L01 required specific media and pH levels to prduce spore as required for developing the bio-agent formulation.

Crude extract test against pathogen

It revealed that crude methanol of *E. nidulans* isolate L01 at 1000 µg/ml was significantly inhibited *F. oxysporum* f sp *lycopersici* 84.40 %, and followed by crude ethyl acetate and crude hexane which were 64.40 and 60.28 %, respectively. Crude methanol of *E. nidulans* isolate L01 expressed antifungal activity against *F. oxysporum* f sp *lycopersici* VTS16 at the ED_{50} of 112 µg/ml, and follwed by crude ethyl acetate and crude hexane which were 379 and 915 µg/ml, respectively (Table 3).

Testing bio-agent formulations to control Fusarium wilt of tomato

Disease index in oil based formulation produced from *E. nidulans* isolate L01 gave the lowest wilt incidence (DSI 1.75) of tomato caused by *F. oxysporum* f sp *lycopersici* VTS16 and followed by powder based formulation (DSI 2.00), culture filtrate (DSI 2.75) and prochoraz (DSI 3.50) when compared to inoculated control (DSI 4.75). It is shown that oil based formulation showed significantly better plant height (119.25 cm) than powder based formulation which plant height was 109.75 cm and followed by culture filtrate and prochoraz which plant height were 84.50 and 73.00 cm, respectively when compared to the inoculated control (62.75 cm). Powder and oil based formulations gave the plant weight of 91.75 and 98.50 g/plant which better in plant weight than culture filtrate and prochoraz (68.00 and 67.00 g/plant) which non-significantly differed when compared the inoculated control (64.00 g/plant). Results in root weight and fruit number/plant were similar to those in plant weight. Tomato var Sida treated with oil and powder based formulation *E. nidulans* isolate L01 gave the highest yields (fruit weight) of 218.50 and 197.50 g/plant, respectively and followed by treated with culture filtrate and prochoraz which yielded 128.00 and 107.00 g/plant, respectively when compared to the inoculated control (83.75 g/plant) as shown in Table 4. As a result, it is indicated that power and oil based formulations increased in plant growth parameters 30-60 % when compared to inoculated control. Oil and powder based formulations reduced the wilt incidence of 63.15 and 57.89 % and followed by culture filtrate and prochoraz which reduced wilt incidence of 42.10 and 36.31 %, respectively as shown in Table 5.

Discussion

F. oxysporum f. sp. *lycopersici* VTS16 was isolated from infested tomato fields in Lao PDR and proved to be the most virulent isolate causing wilt of tomato var. Sida as confirmed by Sibounnavong *et al.* (2010). It is shown that *E. nidulans* isolate L01 which cultured in PDB at pH 8 and mixed media of PDB and CWDB at pH6 produced more fungal biomass which gave highly significant in fresh weight of fungal biomass than other treatments. This was similar to the report of Sibounnavong *et al.* (2009b) who reported that the mixed medium between PDB and CWBD at pH levels 5-8 are suitable for culture of *E. nidulans*. This result suggested that mixed media between PDB and CWDB at pH levels range from 5 - 8 are most optimum medium and pH levels for the growth of *Emericella* spp. than other treatments.

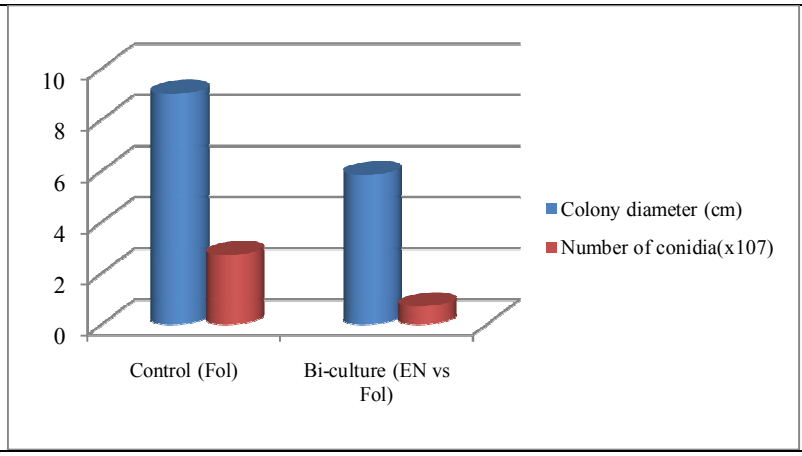
In this research finding, crude methanol of *E. nidulans* isolate L01 at 1000 µg/ml significantly inhibited *F. oxysporum* f sp *lycopersici* 84.40 %, and followed by crude ethyl acetate and crude hexane which were 64.40 and 60.28 %, respectively. These results are reportedby Soytong *et al.* (2005) who stated that the potentail antagonistc fungi would produce some antagonistic substances to express antifungal activity against plant pathogens, and reported that antagonistic substances from *C. globosum* CG extracted with ethyl acetate and *T. harzianum* PC01 extracted with ethyl acetate at 500 µg/ml could inhibit conidia production of *Colletotrichum gloeosporioides* WMF01 causing grape anthracnose at ED_{50} of 2 and 7 µg/ml, respectively. The result of this study showed that crude methanol of *E. nidulans* isolate L01 expressed antifungal activity against *F. oxysporum* f sp *lycopersici* at the ED_{50} of 112 µg/ml, and follwed by crude ethyl acetate and crude hexane which were 379 and 915 µg/ml, respectively. The tested antagonistic fungus showed antibiotic mechanism to inhibit growth of Fusarium wilt pathogen which was supported by Soytong (1992) who stated that antagonistic substance from *C. cupreum*, *C. globosum* and *T. harzianum* could inhibit the growth and break the cells of *F. oxysporum* f.sp. *lycopersici*. Moreover, Park *et al.* (2005) stated that liquid culture of *C. globosum* F0142 could suppress the development of disease more than 80% and can exhibit antifungal activity against *Phytophthora infestans* in tomato at moderate level *in vivo*. However, the related species of *Emericella rugulosa* reported to produce Prenylxanthones and a bicycle(3.3.1) nona-2,6-diene that expressed against human pathogens (Moosophon, *et al*., 2009). However, Moosophone *et al.* (2006) reported that the separation of crude hexane and ethyl acetate extracts from *Emericella nidulans* isolate EN01 yielded six compounds, epishamixanthone (1), shamixanthone (2), emericellin (3), ergosta-6, 22-diene-3-ol-5, 8-epidioxy-(3β- 5α, 22*E*) (4), sterigmatocystin

(5) and demethylsterigmatocystin (6). With this, some of these pure compounds expressed antifungal activity against plant pathogens.

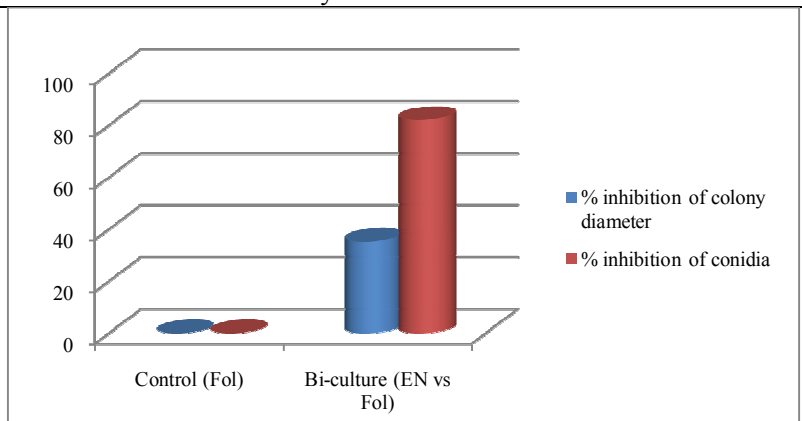
As a result, it is indicated that power and oil based formulations of *E. nidulans* L01 increased in plant growth parameters 30-60 % when compared to inoculated control. The oil and powder based formulations reduced the wilt incidence of 63.15 and 57.89 % and followed by culture filtrate and prochoraz which reduced wilt incidence of 42.10 and 36.31 %, respectively.

The bio-agent formulations of powder and oil forms produced *E. nidulans* are clearly demonstrated effective control of Fusarium wilt of tomato caused by *F. oxysporum* f. sp *lycopersici*. Kaewchai *et al.* (2009) stated that mycofungicide produced from potential effective antagonists gave a good control of plant disease as the same alevel as chemical fungicides. However, Charoenporn *et al.* (2010) reported that oil bio-agent formulation *Chaetomium globosum* and *Ch. lucknowense* showed their biological ability to control tomato wilt cause by *F. oxysporum* f. sp *lycopersici*. The result of bio-agent formulation from *E. nidulans* L01 is similarly reported by Soytong (1992) as stated that registered bio-fungicide formulated from *C. cupreum* could decrease disease incidence of tomato wilt caused by *F. oxysporum* f. sp *lycopersici* and also increased in yield. Soytong et al. (2001) who showed that the biological products consist of *Chaetomium* sp. (22 strains of *C. cupreum* and *C. globosum*) in biopellet and biopowder formulations which when applied to the soil could suppress the growth of *F. oxysporum* f.sp. *lycopersici* and reduced infection rate in tomato. The effective of powder based bio-agent formulations also supported the previous work of Soytong *et al.* (2005) who stated that *Chaetomium* bio-products formulated from *C. globosum* and *C. cupreum* as powder formulation gave a good control Bottle palm rot caused by *Thielaviopsis paradoxa* in the field and reduced disease incidence of 75%.

It is concluded that this reaserch finding of *E. nidulans* isolate L01 developed as bio-agent formulation would be feasible to extend this biological fungicide to control tomato wilt in different tomato varieties where susceptible wilt incidence, especially in the field.



A = colony and number of conidia



B= per cent inhibition of colony and conidia

Fig. 3. Bi-culture antagonistic test between *Emericella nidulans* L01 and *Fusarium oxysporum* f.sp. *lycopersici* VTS16

Table 1. Isolates of *Fusarium oxysporum* f. sp. *lycopersici* and their pathogenicity group

Isolates	Samples	DSI[1]	Pathogenicity group
VTS16	Rhizosphere soil	5.25 a[2]	High
VTS17	Rhizosphere soil	4.25 b	Medium
VTS01	Root	2.37 c	Low
VTS02	Root	2.37 c	Low
VTS03	Root	2.37 c	Low
VTS04	Root	2.12 cd	Low
VTS05	Root	1.87 cde	Low
VTS06	Root	1.87 cde	Low
VTS07	Root	1.75 cde	Low
VTS08	Root	1.62 cde	Low
VTS09	Root	1.62 cde	Low
VTS10	Root	1.62 cde	Low
VTS11	Root	1.50 cde	Low
VTS12	Rhizosphere soil	1.50 cde	Low
VTS13	Rhizosphere soil	1.50 cde	Low
VTS14	Rhizosphere soil	1.50 cde	Low
VTS15	Rhizosphere soil	1.50 cde	Low
VTS18	Rhizosphere soil	1.50 cde	Low
VTS19	Rhizosphere soil	1.25 de	Low
VTS20	Rhizosphere soil	1.00 e	Availurent
CV(%)	-	24.00	-

[1]Disease severity index (DSI) was scored at 21 days after inoculation. 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21-40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting or die 81-100%.

[2]Average of four replications. Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.01.

[3] Pathogenicity group of tested isolates was categorized as Avirulent (A), Low (L), medium (M) and high virulent.

Table 2. Fresh weight of fungal biomass of *E. nidulans* in different liquid media and pH levels

Media	pH	Fresh weight (g)
PDB	5	1.22c
	6	1.26 bc
	7	1.14cd
	8	1.57 a
CWDB	5	0.49f
	6	0.58f
	7	0.54f
	8	0.64f
PDB:CWDB	5	0.87e
	6	1.42 ab
	7	1.29 bc
	8	0.99de
CV(%)		9.1

[1]Average of four replications . Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.01.

Table 3. Bioassay of crude extracts from *E. nidulans* at different concentrations to inhibit *Fusarium oxysporum* f.sp. *lycopersici* isolate VTS16 at 7 days.

Crude extracts	Concentrations (µg/ml)	Number of conidia ($x10^7$)	Conidial inhibition (%)	ED_{50} (µg/ml)
Crude hexane	0	14.6ab	-	
	10	14.07b	4.03i	
	50	12.78c	12.88h	
	100	11.91d	18.8g	915
	500	10.11f	31.1ef	
	1000	5.82h	60.28c	
Crude EtoAC	0	15a	-	
	10	12.31cd	17.9gh	
	50	11.11e	25.88f	
	100	10.11f	32.55e	379
	500	7.13g	52.40d	
	1000	5.33h	64.40c	
Crude MeoH	0	14.10b	-	
	10	11.81d	16.16gh	
	50	10.08f	28.39ef	
	100	5.70h	59.46c	112
	500	3.77i	73.17b	
	1000	2.61j	84.44a	
CV (%)		3.59	7.07	

[1]Average of four replications. Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.01.

Table 4. Testing bio-agent formulations to control Fusarium wilt of tomato *in vivo*

Treatments	Plant height (cm)	Plant weight (g)	Roots weight (g)	Numbers of fruit/plant	Yield	DSI[1]
non inoculated control	101.75c	78.00b	7.25abc	13.50a	155.50b	1.00e
inoculated control	62.75f	64.00b	5.00c	8.00b	83.75d	4.75a
powder formulation	109.75b	91.75a	11.25a	13.25a	197.50a	2.00 cd
Oil formulation	119.25a[2]	98.50a	10.00ab	15.00a	218.50a	1.75de
culture filtrate	84.50d	68.00b	6.00 bc	8.50b	128.00bc	2.75bc
prochoraz	73.00e	67.00b	7.75 abc	9.75b	107.00cd	3.50b
C.V.(%)	3.21	8.03	22.58	11.80	9.16	16.34

[1]Disease severity index (DSI) was scored at 21 days after inoculation. 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21-40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting or die 81-100%.
[2]Average of four replications. Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.01.

Table 5. Increased in percentage of growth parameters from testing bio-agent formulations to control Fusarium wilt of tomato *in vivo*

Treatments	Plant height[1]	Plant weigh	Roots weight	Numbers of fruit/plant	Yield	DR[2]
Powder formulation	42.82	30.24	55.55	39.62	57.59	57.89
Oil formulation	47.37	35.02	50.00	46.66	61.67	63.15
culture filtrate	40.37	5.88	16.66	5.88	34.60	42.10
prochoraz	14.04	4.47	35.48	17.94	21.72	26.31

[1]Increased in plant growth parameters = inoculated control – treatment / inoculated control X 100.

[2]Disease reduction (DR) = disease index of inoculated control - disease index of treatment/disease index of inoculated control X 100.

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