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# Two Edible Mushrooms' Interaction against Fusarium Wilt Which caused by *F. Oxysporum f. sp. Lycopersici*

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**Abstract** The majority of mushrooms are edible, medicinal or health care value, development value is high. The two collected specimens *Clitocybe* spp AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3 were from the rain forest which located in Kanchanaburi Province, Amphoe Mueang Kanchanaburi, Thailand (N 14°0′12″, E 99°33′0″). Crude extracts were yielded from the two specimens. Results showed that the crude hexane, crude ethyly acetate and crude methanol from *Clitocybe* spp AJ2-2 yielded 5.92, 5.48 and 5.99%, respectively. The crude hexane, crude ethyly acetate and crude methanol from *B. affinis* var. *maculosus* AJ2-3 yielded 0.43, 0.47 and 5.32 %, respectively. The crude extarcts from *Clitocybe* sp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 were selected for bioactivity test against fusarium wilt which caused by *F. oxysporum f. sp. lycopersici.* Results showed that crude ethyly acetate from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 83.90 % for spore production of *F.oxysporum* at concentration of 1000 ppm. Crude hexane from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 76.91 % for the spore production of *F.oxysporum* at the concentration of 1000 ppm. These investigations are also reported for the first that time that *Clitocybe, B. affinis* var. *maculosus* and have shown some antimicrobial substances against fusarium wilt which canused by *F.oxysporum*. Further investigation would be studies on chemical elucidation of these antagonistic substances.

**Keywords:** Agaricales, Edible mushroom, *Fusarium.oxysporum.*

## Introduction

Agaricales comprises the so-called mushrooms and toadstools, and is the largest clade of mushroom-forming fungi. More than 9000 species in more than 300 genera, and 26 families had been described. Mostly they are terrestrial, lignicolous and saprobic, and many are mycorrhizal (Kirk *et al*., 2001).

Agaricales belongs to Eumycota、Basidiomycotina Hymenomycetes、Homobasidiomycitidae (Alexopoulos and Mimx, 1979). Mycelium typically formed by the spores germinate primary hyphae, with primary anastomosis

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affinity forming secondary hyphae, no or a lock-like joint, with a septum and barrel bung hole cover. Some types of secondary mycelium may also be formed streptozotocin. Fruiting body is fleshy, easily broken, rarely membranous or leathery. Typical fruiting bodies, including the cap, stipe, located below the cap gills or bacteria tube, located in the middle or upper part of stipe mushroom ring and base volva. Hymenium often in the initial stage of growth within the biofilm shedding covered completely exposed when ripe. No burden separated single spore spores, colorless or colored, its shape, size, color and ornamentation is an important basis for minutes (Arora, 1986).

Agaricales including Pleurotaceae, Schizophyllaceae, Hygrophoraceae, Tricholomataceae, Amanitaceae, Pluteaceae, Agaricaceae, Coprinaceae, Bolbitiaceae, Strophariaceae, Cortinariaceae, Crepidotaceae, Entolomataceae, Paxillaceae, Gomphidiaceae, Boletacea, Russulaceae and others. *Agaricus* L. belongs to Agaricaceae. The majority of mushrooms are edible, medicinal or health care values. For example, *Agaricus bisporus* (Jelange) Imbach, occurs scaly mushrooms, *Agaricus crocopeplus* Berk, woodland mushrooms, *Agaricus silvaticus* Schaeff, large purple mushroom, *Agaricus augustus* Fr, white mushrooms, *Agricus bernardii* (Qué.) Sacc, big fat mushrooms, *Agaricus bitorquis* (Qué.) Sacc, and the four spore mushrooms as *Agaricus campestris* L. Which has been carried out in artificial cultivation in order for edible, *Agaricus subrufescens* Peck reported to do liquid fermentation and mycelia contains large amounts of polysaccharides and other biologically active substances (Genpei Yu and Jigui Bao, 2008), *Agaricus arvensis* Schaeff reported that involved in the human body's immune system regulating function has the good role in promoting, aroused people's great concern to the wild mushrooms. Brazil mushrooms *Agaricus blazei* Murr reported to be involved in lowering blood sugar, improved arteriosclerosis and cytotoxicity to some cancer cell lines (Xiaoping Luo and Junyan Wang 2007). The seasonal climate of Thailand coupled with the complex topography has resulted in rich biodiversity, including of fungal diversity. The objective of this research project was preliminary conduced to test extracted biological activity substances that inhibit plant pathogens-*Fusarium oxysporum*.

Tomatoes have bleeding, blood pressure, diuretic, stomach and digestion, thirst, detoxification effect. Since the ratio of tomato vitamin A, vitamin C suitable, so eat can enhance the function of small blood vessels, prevent vascular aging. Tomato flavonoids, both reducing capillary permeability and prevent rupture of the role, as well as the prevention of hardening of the arteries of the special effects that can prevent cervical cancer, bladder cancer and pancreatic cancer and other diseases; Tomatoes help flattening wrinkles, make the skin smooth and delicate, inhibit bacteria. Eat

tomatoes also less prone to dark circles, and not susceptible to sunburn. There are also many diseases on tomatoes. Especially *Fusarium oxysporum* f. sp. *lycopersici*, the fungus that causes fusarium wilt, attacks only certain tomato cultivars. Plants infected by this soil-dwelling fungus show leaf yellowing and wilting that progress upward from the base of the stem. Initially, only one side of a leaf midrib, one branch, or one side of a plant will be affected. The symptoms soon spread to the remainder of the plant (Fig.1). Wilted leaves usually drop prematurely. Affected plants die early and produce few, if any, fruits. Splitting open an infected stem reveals brownish streaks extending up and down the stem (Fig. 2). These discolored streaks are the water-conducting tissue, which becomes plugged during attack by the fungus, leading to wilting of the leaves. Plants are susceptible at all stages of development, but symptoms are most obvious at or soon after flowering. To minimize losses from Fusarium wilt, it is advisable to plant resistant varieties, and many resistant varieties are available. The letter "F" following the variety name indicates resistance to one or more races of the Fusarium fungus. Resistant varieties may become infected, but disease will not be as severe as with susceptible varieties and a reasonable yield should still be obtained. In addition, plant disease-free seed or transplants in well drained, disease-free soil, rotate at least four years away from tomatoes to reduce populations of the fungus in soil, and remove and destroy infected plant residue. In greenhouse or seedbeds, disinfest soil by treating with steam.

**Figure 1.** Fusarium wilt

**Figure 2.** Vascular browning caused by Fusarium wilt

## Materials and methods

### *Collection and identification*

Mushroom samples were collected during the raining season. Each collection site was recorded the macroclimates, chemical test and photograph of fresh specimens. Spore prinit was done as necessary in the collection sites. The specimens were brought to laboratory for further works, imorphologically identification and isolation to pure cultures.

### *Isolation of Pathogen and Pathogenicity Test*

*Fusarium oxysporum* f. sp. *lycopersici* causing witl of tomato *Lycopersicon esculentum* was isolated from root symptom by tissue transplanting techniques. The cultures were transferred into Potato Dextrose Agar (PDA) and incubated at room temperature. The morphological characteristic of *Fusarium* was studied under compound microscope. The pathogenicity test of fusarium wilt (*Fusarium oxysporum f. sp. lycopersici* NKSC02 race 2) was conducted in vivo to 15 day tomato seedlings. Tomato seeds were sown into coarse sand in plastic trays (10 × 15 × 5 cm) and maintained for 2 weeks. Pathogenicity test was carried out using a rootdip inoculation method. Tomato seedlings were uprooted gently and roots were washed with tap water to remove all sand (Bao J.R. *et al.,* 2001). The spore suspension for inoculation was prepared by pouring 50 ml of sterile water into each of Petri dishes containing 10-day-old Fusarium isolate, stirring the mixture with a sterile glass stick, and pouring it into a glass. The concentration of conidia in the suspension was determined using Haemacytometer to adjust the number of spores to $1x10^6$ conidia/ml. The 3-4 root tips will be cut and soaked into spore suspension for 30 seconds. Control plants were sown in soil and and

be treated with sterile distilled water. Incubation was performed at 22-25 ℃ for 14 days. The disease severity was rated with follow the method of Sibounnavong (2012), 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.

***Extraction of Biological Active Substances***

The bioactive compounds were cultured in potato dextrose broth (PDB) at room temperature (28-30 C) for 45 days and extracted from the collected species of Agaricales as crude extracts. Fungal biomass were collected by moving from PDB, filtered through cheesecloth and air-dried overnight. Fresh and dried fungal biomass was recorded. Dried fungal biomass were ground with electrical blender, extracted with 200 ml hexane (H) and shaken for 5 days at room temperature. The filtrate from ground biomass was separated by filtration through Whatman No.4 filter paper. The filtrate was evaporated in *vacuum* to yield crude extract. The marc was further extracted with ethyl acetace (EtOAc) and methanol (MeOH) respectively using the same procedure as hexane. Each crude extract was weighted, and then kept in refrigerator at 4 C until use.

***Bioactivity against tomato wilt which caused by Fusarium oxysporum f. sp. lycopersici***

The crude extracts were tested for inhibition of the most aggressive isolat *C. coffeanum*. The experiment was conducted by using 3x6 factorials in Completely Randomized Design (CRD) with four replications. Factor A represented crude extracts which consisted of crude hexane, crude ethyl acetate and crude methanol and factor B represented concentrations 0, 10, 50, 100 and/or 500, and 1,000 µg/ml. Each crude extract was dissolved in 2% dimethyl sulfoxiden (DMSO), mixed into potato dextrose agar (PDA) before autoclaving at 121C, 15 1bs/inch2 for 30 minutes. The tested pathogen were cultured on PDA and incubated at room temperature for 5 days, and then colony margin was cut by 3 mm diameter sterilized cork borer. The agar plug of pathogen was transferred to the middle of PDA plate (5.0 cm diameter) in each concentration and incubated at room temperature (28-30C) for 5 days. Data were collected as colony diameter and computed the percentage of inhibition. Data were statistically computed analysis of variance. Treatment means were compared with DMRT at P=0.05 and P=0.01.

## Results

### *Collection and Identification*

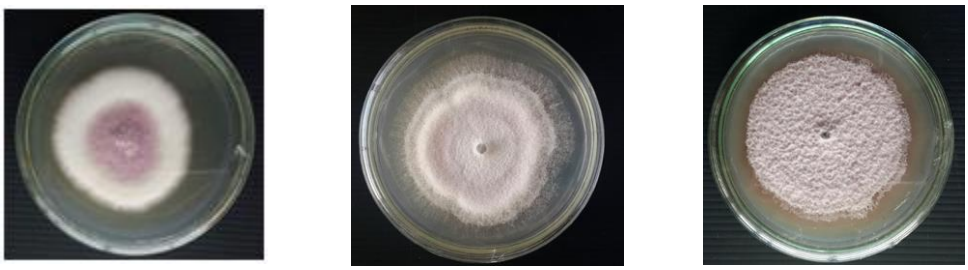
Two specimens were collected from Thailand. They are *Clitocybe* spp AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3, described as follows: ***Clitocybe* spp AJ2-2**: Cap 0.5-7 cm across, purperish to pink to pale brown, horn with strongly depress in the center and inrolled margin becoming wavy. Gills are decurrent, white to olive-yellow. Stem *3.5*-9 cm, cylindrical, smooth, and pink to dark brown. Habitat grows in clusters (Fig. 3). ***Boletus affinis* var. *maculosus* AJ2-3:** Cap 1-3.5 cm across, velvety redish-brown, dry shin, having a membraneous vein on the top part which promptly turns to tobacco color due to the falling spores. Gills are adnate, white. Stem is *6*-9 cm long, cylindrical, silky membranous, smooth. Habitat grows in clusters (Fig.4).

**Figure 3.** *Clitocybe* spp

**Figure 4.** *Lactarius sp*

### *Isolation of pathogen and pathogenicity test*

With the tissue transplanting techniques, the pure cultures and spores of *F.oxysporum* were got, shown as fig. 5 and fig.6, respectively. After performing pathologenicity test followed Koch's Postulate 14 days, there were obvious sysmptoms on tomato. After careful observation, it was found that tomotoes which inoculated in spore suspension of F.oxysporum before planting wilted compared with non-treated group (Fig. 7).



5 days 10 days 15 days

**Figure 5.** Pure cultures of *F.oxysporum*

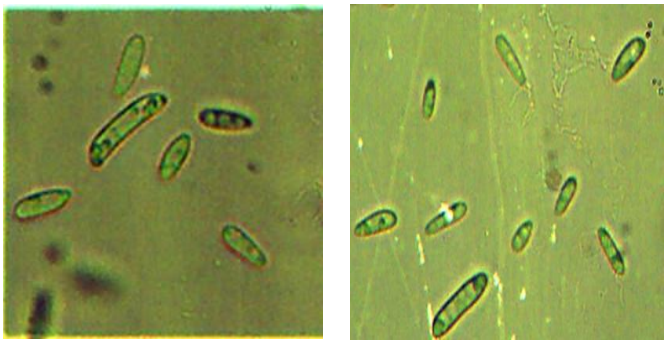


**Figure 6.** Spores of *F.oxysporum*

**Figure 7.** Pathologenicity test

Where: C-Control (root soaked into sterilized water) T-Test (root soaked into spore suspection -$10^6$)

### *Extraction of Biological Active Substances*

Each fungal biosmass was separately extracted to get crude hexane, crude ethyly acetate and crude methanol. The crude hexane, crude ethyly acetate and crude methanol from *Clitocybe* spp AJ2-2 yielded 5.92, 5.48 and 5.99%, respectively. The crude hexane, crude ethyly acetate and crude methanol from *B. affinis* var. *maculosus* AJ2-3 yielded 0.43, 0.47 and 5.32 %, respectively.

### *Bioactivity against Fusarium oxysporum*

The crude extarcts from *Clitocybe* sp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 were selected for bioactivity test against fusarium wilt caused by *F. oxysporum*. Results showed that methanol crude extract from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 27 % for the colony growth of *F. oxysporum* at the concentration of 1,000 ppm when compared to the control (Table 1). Crude ethyl acetate from *Clitocybe* sp AJ2-2 gave significantly highest inhibited the spore production of *F. oxysporum* as 83.90 %, followed by crude methanol inhibited 77.68 % and crude hexane 68.95 % (Tables 2). The ethyl acetate crude extract from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 35.50 % for the colony growth of *F. oxysporum* at the concentration of 1,000 ppm when compared to the control (Table 3). Crude ethyl acetate from B. affinis var. maculosus AJ2-3 gave significantly highest inhibited the spore production of *F. oxysporum* as 79.71 % at the concentration of 1,000 ppm , followed by crude hexane inhibited 76.91% and crude methanol inhibited 64.36 % (Table 4).

**Table 1.** Crude extracts of *Clitocybe sp* Aj2-2 testing for growth inhibition of *Colletotrichum coffaenum* at 5days

| Crude extracts | Concentration (ppm) | Colonydiameter (cm)[1] | Growth inhibition(%)[2] |
|---|---|---|---|
| Crude Hexane | 0 | 5.00[a] | 0.00[j] |
| | 10 | 5.00 [a] | 0.00[j] |
| | 50 | 5.00 [a] | 0.00[j] |
| | 100 | 4.90 [a] | 2.00[i] |
| | 500 | 4.80[b] | 4.00[h] |
| | 1000 | 4.62[c] | 7.50[g] |
| Crude EtOAc | 0 | 5.00 [a] | 0.00[j] |
| | 10 | 4.35[de] | 13.00[ef] |
| | 50 | 4.27ef | 14.50[de] |
| | 100 | 4.20[f] | 16.00[d] |
| | 500 | 4.10g | 18.00[c] |
| | 1000 | 4.00h | 20.00[b] |

| | | | |
|---|---|---|---|
| Crude MeOH | 0 | 5.00 [a] | 0.00[j] |
| | 10 | 4.40[d] | 12.00[f] |
| | 50 | 4.27[ef] | 14.00[e] |
| | 100 | 4.10[g] | 18.00[c] |
| | 500 | 4.00[h] | 20.00[b] |
| | 1000 | 3.65[i] | 27.00[a] |

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

**Table 2.** Spore production inhibition of crude extracts from *Clitocybe* sp AJ2-2 to *Fusarium oxysporum* f sp *lycopersici* at 7days

| **Crude extracts** | **Concentration (ppm)** | **Number of spores $^{1}(10^{6})$** | **Inhibition (%)[2]** | **$ED_{50}$** |
|---|---|---|---|---|
| Crude Hexane | 0 | 132.63[a] | 0.00 [i] | *409.59* |
| | 10 | 114.75[b] | 13.47[h] | |
| | 50 | 107.81[b] | 18.72[h] | |
| | 100 | 88.88[c] | 33.01[g] | |
| | 500 | 72.19[de] | 45.58[f] | |
| | 1000 | 0.25[b] | 68.95c | |
| Crude EtOAc | 0 | 132.63[a] | 0.00 [i] | 17.54 |
| | 10 | 67.25[de] | 49.3[ef] | |
| | 50 | 56.14[fg] | 57.68[d] | |
| | 100 | 56.31[fg] | 57.54 [d] | |
| | 500 | 0.81[b] | 68.36[c] | |
| | 1000 | 21.38[ij] | 83.90[a] | |
| Crude MeOH | 0 | 132.63[a] | 0.00 [i] | 26.43 |
| | 10 | 75.25[d] | 43.32[f] | |
| | 50 | 63.50[ef] | 52.11[de] | |
| | 100 | 48.3[gh] | 52.11[de] | |
| | 500 | 29.63[i] | 63.59[c] | |
| | 1000 | 16.88[j] | 77.68[b] | |
| C.V.(%) | | 3.05 | 31.43 | |

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

**Table 3.** Crude extracts of *Boletus affinis* var. *maculosus* AJ2-3 testing for growth inhibition of *Fusarium oxysporum* f sp *lycopersici* at 5days

| Crude extracts | Concentration (ppm) | Colonydiameter (cm)[1] | Growth inhibition(%)[2] |
|---|---|---|---|
| Crude Hexane | 0 | 5.00a | 0.00d |
| | 10 | 5.00a | 0.00d |
| | 50 | 5.00a | 0.00d |
| | 100 | 5.00a | 0.00d |
| | 500 | 5.00a | 0.00d |
| | 1000 | 5.00a | 0.00d |
| Crude EtOAc | 0 | 5.00a | 0.00d |
| | 10 | 5.00a | 0.00d |
| | 50 | 5.00a | 0.00d |
| | 100 | 4.45b | 11.00c |
| | 500 | 4.25c | 15.05b |
| | 1000 | 3.22d | 35.50a |
| Crude MeOH | 0 | 5.00a | 0.00d |
| | 10 | 5.00a | 0.00d |
| | 50 | 5.00a | 0.00d |
| | 100 | 5.00a | 0.00d |
| | 500 | 5.00a | 0.00d |
| | 1000 | 5.00a | 0.00d |
| CV(%) | | 1.12 | 0.83 |

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.
[2]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

**Table 4.** Spore production inhibition of crude extracts from *Boletus affinis* var. *maculosus* AJ2-3 to *Fusarium oxysporum* f sp *lycopersici* at 7 days

| Crude extracts | Concentration (ppm) | Number of spores[1] ($10^{x6}$) | Inhibition(%)[2] | $ED_{50}$ |
|---|---|---|---|---|
| Crude Hexane | 0 | 83.00[a] | 0.00[i] | 151.44 |
| | 10 | 71.75[b] | 13.44[h] | |
| | 50 | 50.69[ef] | 35.11[f] | |
| | 100 | 53.81[fde] | 46.85[e] | |
| | 500 | 32.50[hi] | 60.89[bc] | |
| | 1000 | 19.25[i] | 76.91[a] | |

| | | | | |
|---|---|---|---|---|
| Crude EtOAc | 0 | 83.00[a] | 0.00[i] | 59.85 |
| | 10 | 44.94[fg] | 45.90[e] | |
| | 50 | 41.50[g] | 49.84[de] | |
| | 100 | 39.13[c] | 52.86[de] | |
| | 500 | 28.63[i] | 65.50[b] | |
| | 1000 | 16.88[j] | 79.71[a] | |
| Crude MeOH | 0 | 83.00[a] | 0.00[i] | 131.90 |
| | 10 | 65.13[bc] | 21.45[g] | |
| | 50 | 59.06[cd] | 28.79[f] | |
| | 100 | 38.19[gh] | 28.79[f] | |
| | 500 | 29.63[i] | 54.29[cd] | |
| | 1000 | 19.50[j] | 64.36[b] | |
| C.V.(%) | | 3.05 | 7.79 | 9.12 |

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

## Discussion

As results showed that methanol crude extract from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 27 % for the colony growth of *F. oxysporum* at the concentration of 1,000 ppm, crude ethyl acetate gave significantly highest inhibited the spore production as 83.90 %, the effective dose ($ED_{50}$) could inhibit the spore growth of *F. oxysporum* at the concentrations of 17.54 ppm. The ethyl acetate crude extract from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 35.50 % for the colony growth of *F. oxysporum* at the concentration of 1,000 ppm, spore production as 79.71 % , and the effective dose ($ED_{50}$) could inhibit the spore growth of *F. oxysporum* at the concentrations of 59.85 ppm. In the literature reviews Phillips (1991) and States (2004) cited the two species *Clitocybe* sp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 were described which have been found in Thailand. The research findings are reported for the first time that the metabolites from Clitocybe sp AJ2-2 and B. affinis var. maculosus could inhibit *C. coffaenum* causing coffee anthracnose. Similar report from Badalyan *et al.* (2002) stated that the antagonistic activity of 17 species of Basidiomycotina (*Coriolus versicolor, Flammulina velutipes, Ganoderma lucidum, Hypholoma fasciculare, H. sublateritium, Kühneromyces mutabilis, Lentinula edodes, Lentinus tigrinus, Pholiota alnicola, Ph. aurivella, Ph. destruens, Pleurotus ostreatus, P. cornucopiae, Polyporus squamosus, P. subarcularius, P. varius* and *Schizophyllum commune*) could inhibit plant pathogens, *Bipolaris*

*sorokiniana, Fusarium culmorum, Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia cerealis* that causing foot and root diseases of winter cereals. The potential of fungal metabolites from fungi have been usually reported to produce antibiotic substances against human and plant pathogens. Phoutthasone Sibounnavong (2012) reported that *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. 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. Crude methanol of *E. nidulans* isolate L01 expressed antifungal activity against *F. oxysporum* f sp *lycopersici* at the ED50 of 112 µg/ml, and follwed by crude ethyl acetate and crude hexane which were 379 and 915 µg/ml, respectively.

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