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Full Length Research Paper

The role of *Emericella rugulosa* as a bio-control agent for controlling Fusarium wilt of tomato

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***Fusarium oxysporum* f.sp. *lycopersici* isolate NKSC02 showed the highest virulence for wilt incidence of tomato var Sida. Culture of the antagonistic fungus *Emericella rugulosa* on PDB+CWDB at pH 5 - 8 gave the highest significant fresh weight of fungal biomass which served as an appropriate medium for increasing the number of spores that produces bioformulation. The ED_{50} of crude ethyl acetate from *E. rugulosa* against *F. oxysporum* f.sp. *lycopersici* isolate NKSC02 was 138 µg/ml, while crude hexane and crude methanol were 313 and 1372 µg/ml, respectively. Tajixanthone, a pure compound of *E. rugulosa*, expressed antifungal activity against mycelial growth, macrocondia and microconidia of *F. oxysporum* f. sp. *lycopersici* NKSC02 with ED_{50} of 122, 54 and 42 µg/ml, respectively. It implies a role of antibiosis. Disease immunity to Fusarium wilt in Sida variety showed the highest immunity of 80.95% when treated with crude EtoAC at 1000 µg/ml, followed by crude EtoAC at 500 µg/ml, with an immunity of 30.09%. Bio-formulations produced from *E. rugulosa* in powder and oil bases gave highly significant difference in disease severity index (DSI) when compared to the non-treated control. Oil based bioformulation gave higher significant growth parameters and yield than powder based formulation, culture filtrate of *E. rugulosa* and chemical fungicide treatment; it also lowered wilt incidence of tomato than the others.**

Key words: *Emericella rugulosa*, *Fusarium oxysporum* f. sp. *lycopersici*, crude extracts, tajixanthone, bioformulation.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely cultivated, popular, and important vegetable crops in the world. *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen is one of the most common pathogen that causes wilt of tomato in upland cultivation areas leading to economic losses. *F. oxysporum* f. sp. *lycopersici* has become one of the most damaging and difficult to control wherever tomatoes are grown intensively because it grows endophytically and persists in infested soils (Agrios, 1997). The disease control measures for this vascular wilt are either inefficient or application of the chemical fungicides is difficult. Over time, tomatoes may develop resistance to some races of the pathogen; however, the pathogenic fungus may also develop resistance to chemical fungicides (Silva and Bettiol, 2005). *Emericella* spp. belongs to the Ascomycota and has been reported as an antagonist against plant pathogen (Sibounnavong et al., 2010). *Emericella rugulosa* used in this study has been shown to produce five prenylxanthones, ruguloxanthones A–C, 14-methoxytajixanthone,-tajixanthone ethanoate, a bicyclo[3.3.1] –nona-2,6-diene derivative named rugulosone, shamixanthone, tajixanthone, 14-methoxytajixanthone-25-acetate, taji-

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xanthone hydrate, tajixanthone methanoate, isoemericellin and ergosterol. Among these, the bicyclo[3.3.1]–nona-2,6-diene derivative has been shown to exhibit antimalarial, antimycobacterial activity and cytotoxicity against three cancer cell lines (Moosophon et al., 2009). Another species of *Emericella* (*Emericella nidulans*) has also been reported to antagonize *F. oxysporum* f. sp. *lycopersici* (Sibounnavong et al., 2009a, 2010). The antimicrobial activity of tajixanthone, produced by *E. rugulosa* ER01, could be involved in the disease control mechanism of these antagonistic fungi against the tomato wilt fungus *F. oxysporum* f. sp. *lycopersici*. The objectives of this study were to evaluate the antagonistic fungus, *E. rugulosa*, as a new antagonist against *F. oxysporum* f. sp. *lycopersici* causing tomato wilt and to elucidate its control mechanism of antibiosis, as a role of bio-control of wilt disease.

MATERIALS AND METHODS

Pathogen and pathogenicity test

Pure cultures of *F. oxysporum* f. sp. *lycopersici* were isolated from Burirum, Khonkaen, Nongkhai, Nakhonratchasima and Sakon-nakhon provinces in Thailand, and cultured on potato dextrose agar (PDA) as follows:- isolates BRCO3, KK2, KSoC02, NKSC01, NKSC02, NKRC02, NKRC04, NKRC09, NSC01, SRC02, SSoC03, and SSoC04. These isolates were previously confirmed by sequencing the internal transcribed spacer (ITS) region ITS1, 5.8S and ITS2, and a small portion of 18S rDNA and 28S rDNA (Charoenporn et al., 2010). Pathogenicity was reconfirmed by inoculating the pathogen to 15 day old tomato seedling var. Sida using root-dipped method (Marlatt et al., 1996) with conidial suspension of pathogen at 1×10^7 conidia/ml. Disease severity index (DSI) was scored at 21 days after inoculation based on the modified disease severity scale as follows: 1 = no symptom; 2 = plant showing yellow leaves and wilting (1 to 20%); 3 = plant showing yellow leaves and wilting (21 to 40%); 4 = plant showing yellow leaves and wilting (41-60%); 5 = plant showing yellow leaves and wilting (61 to 80%); and 6 = plant showing yellow leaves, wilting (81 to 100%) or death. Pathogenicity test was conducted twice for each isolate. All tested isolates were recorded for pathogenic and non-pathogenic isolates. Pathogenicity group was categorized according to DSI as non-pathogenic (DSI = 1); low (DSI ≤ 3.50); moderate (DSI > 3.50 to 4.50) and high (DSI > 4.50). The most aggressive isolate was selected for further experiment.

Growth of *Emericella rugulosa* ER01 culture in liquid media and pH levels

E. rugulosa ER01 isolated from cultivated soil was used as antagonistic fungus. The fungus was sub-cultured on PDA for examination of the morphological characteristics that will be used in further study. Three media namely potato dextrose broth (PDB), coconut water dextrose broth (CWDB) and mixture of PDB and CWDB (1:1) were prepared and used in this experiment. Potato dextrose broth (PDB) was prepared by boiling 200 g of potato in 1000 ml of water and mixed with 20 g of dextrose; CWDB was prepared by boiling 1000 ml of coconut water mixed with 20 g of dextrose. The media were separated in 20 ml of medium in each flask and the pH levels were adjusted by adding either HCl or NaOH to get the required pH levels; after that, the media were sterilized by autoclaving at 121°C, 15 lbs/inch^2 for 20 min. Then, an agar plug (0.3 cm diameter) of *E. rugulosa* was transferred into each Petri dish and incubated for 14 days at room temperature (approximately, 30 to 32°C). After 14 days, the culture on each Petri dish was separately filtered using Whatman filter paper No. 4 to get the fresh fungal biomass. The fungal biomass was air dried at room temperature for 48 h. Fresh fungal biomass was weighted (g) using electrical balance. The experiment was set up using two factorial experiments in completely randomized design (CRD). The two factors were as follows: three kinds of media and the four pH levels of the media. Each treatment consisted of four replications. The following are the treatments of the study: Factor A - kinds of media a1 = PDB; a2 = CWDB; a3 = mixture of PDB and CWDB; Factor B – pH levels: b1 = 5, b2 = 6, b3 = 7 and b4 = 8. The most suitable medium and pH level for the fungus growth were used as medium to culture the antagonist for preparing the fungal biomass used in formulating the biofungicides for controlling Fusarium wilt of tomato in the pot experiment.

Crude extract bioassay against *F. oxysporum* f. sp. *lycopersici*

Crude extraction from *E. rugulosa* was done by following the method of Kanokmedhakul et al. (2006), Moosophon et al. (2009) and Thohinung et al. (2010).

E. rugulosa was cultivated in potato dextrose broth (PDB) at room temperature for 30 days to yield fresh fungal biomass and dried overnight. The dried fungal biomass was ground and sequentially extracted with hexane, ethyl acetate, and methanol. The crude filtrate was evaporated *in vacuo* to separate solvent and then yielded 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 crude extracts, 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 mixed with PDA before autoclaving at 121°C (15 psi) for 30 min. A sterilized cork borer was used to remove agar plugs from the actively growing edge of the pathogen culture at 3 mm diameter. An agar plug was transferred to the center of 5 cm diameter of each Petri dish on PDA containing crude extract at each concentration and incubated at room temperature 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.

Pure compound bioassay against *F. oxysporum* f. sp. *lycopersici*

Tajixanthone is a pure compound from chromatographic separation of the crude hexane extract from *E. rugulosa*. Its structure was identified by spectroscopic method.

Dried mycelium mat of *E. rugulosa* was ground into powder and then extracted thrice with hexane. The solvent extract was evaporated *in vacuo* to get crude hexane, extract. The crude hexane extract was separated by chromatographic methods. The structures of the isolated compound were elucidated based on IR, ^{1}H NMR, ^{13}C NMR and 2D NMR. The experiment was conducted using CRD with four replications. Treatments were at concentrations of 0 µg/ml (control), 50 µg/ml, 100 µg/ml and ≥500 µg/ml. The method was done the same way as crude extract

bioassay.

Effect of crude extracts for disease immunity of wilt incidence in tomato var Sida

The experiment was conducted using a CRD with four replications. Treatments were conducted as follows: T1= control - non-inoculated with conidia of pathogen; T2= control - inoculated with conidia of the pathogen; T3= inoculated with pathogen mixed with 500 μg/ml of the most effective crude extract and T4= inoculated with pathogen mixed with 1000 μg/ml of the most effective crude extract. The roots of 20-day-old tomato seedlings var. Sida were washed under running sterilized water and cut at five points on the root tips before dipping the roots into each treatment. A 20 ml spore suspension of $1×10^7$ spores/ml was mixed with different concentrations of crude extract for 15 min. The seedlings were then planted in pots which contained sterilized soil. The experiment was repeated twice. DSI was scored as previous experiment and disease immunity (%) was computed as follows:

DSI in control - DSI in treatment/ DSI in control × 100

Testing bioformulation of *E. rugulosa* for controlling Fusarium wilt of tomato

Bioformulations were separately formulated as powder, oil based formulation according to the method of Soytong (2001) by using fungal biomass of *E. rugulosa*. The antagonistic fungus was cultured in the PDB mixed CWDB at pH 5 to 6 and incubated at room temperature for 30 days. The number of antagonistic spores in bioormulation was adjusted to $2 × 10^7$ spores/ml before adding either sterilized palm oil for oil based bioformulation or sterilized talcum for powder based bioformulation.

Bioformulations of *E. rugulosa* were tested for their abilities to control tomato wilt caused by *F. oxysporum* f. sp *lycopersici in vivo*. Tomato seedlings var. Sida at 30 days old were inoculated with conidial suspension of *F. oxysporum* f. sp. *lycopersici* at concentration of $1×10^7$conidia/ml by dipping root for 15 min and transplanting into plastic pot that contained sterilized mixed soil (soil: sand: compost, 4:1:1). Sterilized mixed soil was sterilized at 121°C, 15 lbs/inch^2 for 1 h in two consecutive days. Randomized completely block design (RCBD) was performed 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 from antagonist (T_3), powder bioformulation (T4), oil liquid bioformulation (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 bioformulation and prochoraz 50% WP chemical fungicide 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 height (cm), plant fresh (g), fruit weight (g), fruit per plant and root weight (g). Disease severity index (DSI) was scaled as previous experiment. Percentage of disease reduction was analyzed using the formula:

% disease reduction = (Disease severity index of control – Disease severity index of treatment)/ Disease severity index of control × 100.

Percent increase in yield was analyzed using the formula:

(Yield per plant in treatment – yield per plant in control)/ yield per plant in treatment × 100.

All data were subjected to analysis of variance (ANOVA). Treatment means were statistically compared with Duncan's new multiple range test (DMRT) at $P≤0.05$ to separate means. The experiment was repeated two times.

RESULTS

Pathogen and pathogenicity test

It was shown that the isolate NKSC02 gave the highest significant disease index of 5.50 level which resulted in high virulent wilt of tomato var Sida, followed by isolates NKSC01 and KK2, with DSI of 4.75 and 4.25 respectively (Table 1). The isolates BRC03, KSoC02, NKRC02, NKRC04, NKRC09, NSC01, SRC02, SSoC03 and SSoC04 were non-pathogenic isolates. Isolate NKSC02 was then selected for further experiment.

Growth of *Emericella rugulosa* ER01 culture in liquid media and pH levels

Results showed that *E. rugulosa* could grow very well in mixed media of PDB and CWDB in the range of pH levels 5 to 8, which gave significantly produced fresh weight of fungal biomass at 1.70, 1.57, 1.60 and 1.70 g / Petri dish when compared with other treatments respectively as seen in Table 2. This result suggested that mixed media of potato dextrose broth (PDB) and coconut water dextrose broth (CWDB) at pH levels of 5 to 8 are more suitable for mycelial production of *E. rugulosa* than other treatments. It is concluded that PDB+CWDB at pH 5 to 8 gave significantly highest weight of fresh fungal biomass, followed by PDB at pH 7 to 8 and CWDB at pH 6, respectively.

Crude extract bioassay against *F. oxysporum* f. sp. *lycopersici*

Crude extracts of the antagonist *E. rugulosa* could inhibit conidial production (macroconidia and microconidia) of the pathogen at the concentrations range of 10 to 1000 μg/ml as shown in Table 3. Crude extracts of tested antagonists showed the highest properties to inhibit conidial production of *F. oxysporum* f.sp. *lycopersici* NKSC02 at 1000 μg/ml. Crude ethyl acetate of *E. rugulosa* was the most effective crude extract gave a highly significant different colony diameter and lowest number of conidia production of the pathogen when compared with other treatments which were 3.67 cm and $2.15 × 10^7$ spore/ml; while control plate mixed with the crude extract inhibits conidia produced by the pathogen at $17.6 × 10^7$ spore/ml followed by crude hexane that gave colony diameter of 4.29 cm and 4.22×10^7 spore/ml number of conidia. Crude methanol gave 4.62 cm of colony diameter and 7.54×10^7 spore/ml of conidia. The highest conidial inhibition was presented by crude ethyl

Table 1. Isolates of *Fusarium* spp. and their pathogenicity groups in Sida variety.

Sources	Isolates	DSI[1]	Pathogenicity groups
Burirum	BRC03	1.00[d2]	Non-pathogenic
KhonKaen	KK2	4.25[c]	Moderate virulent
	KSoC02	1.00[d]	Non-pathogenic
Nongkhai	NKSC01	4.75[b]	High virulent
	NKSC02	5.50[a]	High virulent
	NKRC02	1.00[d]	Non-pathogenic
	NKRC04	1.00[d]	Non-pathogenic
	NKRC09	1.00[d]	Non-pathogenic
Nakhonratchasima	NSC01	1.00[d]	Non-pathogenic
Sakon Nakhon	SRC02	1.00[d]	Non-pathogenic
	SSoC03	1.00[d]	Non-pathogenic
	SSoC04	1.00[d]	Non-pathogenic

[1]Disease severity index (DSI) was scored at 21 days after inoculation. 1 = no symptom; 2 = plant showed yellowing leaves and wilting (1 to 20%), 3 = plant showed yellowing leaves and wilting (21 to 40%), 4 = plant showed yellowing leaves and wilting (41 to 60%), 5 = plant showed yellowing leaves and wilting 61-80%, and 6 = plant showed yellowing leaves and wilting or die (81 to 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 was categorized according to DSI as non-pathogenic (DSI = 1), low (DSI ≤ 3.50), moderate (DSI > 3.50 to 4.50), and high (DSI > 4.50).

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

Media	pH	Fresh weight (g)
PDB	5	1.30[cd]
	6	1.19[d]
	7	1.54[ab]
	8	1.29[cd]
CWDB	5	1.14[d]
	6	1.39[bc]
	7	1.15[d]
	8	1.13[d]
PDB:CWDB	5	1.70a[1]
	6	1.57[a]
	7	1.60[a]
	8	1.70[a]
CV (%)		6.57

[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.

acetate at the concentration of 1000 µg/ml followed by crude hexane and crude methanol which were at 87.55, 76, and 55.41%, respectively. Crude ethyl acetate showed the highest inhibition of conidial production of the

Table 3. ED_{50} of crude extracts from *Emericella rugulosa* to inhibit *Fusarium oxysporum* f.sp. *lycopersici* isolate NKSC02 at 7 days.

Crude extracts	Concentrations µg/ml)	Number of conidia x10^7	Conidial inhibition (%)	ED_{50} (µg/ml)
Crude hexane	0	17.60^{a}	-	313
	10	16.67^{abc}	5.12^{h}	
	50	15.45^{def}	12.18^{efg}	
	100	12.33^{g}	29.86^{d}	
	500	7.92^{h}	55.06^{c}	
	1000	4.22^{i}	76.00^{b}	
Crude EtoAC	0	17.50^{ab}	-	138
	10	15.65^{cde}	9.43^{fgh}	
	50	14.47^{ed}	16.03^{ef}	
	100	8.66^{h}	49.75^{c}	
	500	4.50^{i}	73.87^{b}	
	1000	2.15^{j}	87.55^{a}	
Crude MeOH	0	17.00^{ab}	-	1372
	10	16.37^{bcd}	6.43^{gh}	
	50	15.44^{def}	11.6^{efgh}	
	100	14.33^{f}	17.89^{e}	
	500	15.05^{g}	25.26^{d}	
	1000	7.54^{h}	55.41^{c}	
CV (%)		4.77	-	

[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. ED_{50} of tajixanthone, a pure compound of *Emericella rugulosa* to inhibit *Fusarium oxysporum* f.sp. *lycopersici* isolate NKSC02 at 7 days.

Inhibition	ED_{50} (µg/ml)
Colony	122
Macroconidia	54
Microconidia	42

pathogen in which ED_{50} value was 138 µg/ml while crude hexane and crude methanol presented their abilities to inhibit conidial production at the ED_{50} of values 313 and 1372 µg/ml, respectively.

Pure compound bioassay against *F. oxysporum* f. sp. *lycopersici*

A pure compound of tajixanthone from *E. rugulosa* elucidates the structures based on IR, ^{1}H NMR, ^{13}C NMR and 2D NMR. Result confirmed that tajixanthone expressed antifungal activity against mycelial growth, macrocondia and microconidia of *F. oxysporum* f.sp. *lycopersici* NKSC02 with ED_{50} of 122, 54 and 42 µg/ml, respectively (Table 4).

Effect of crude extracts on disease immunity of wilt incidence in tomato var Sida

Result found that treated tomato seedlings var Sida with crude EtoAC of *E. rugulosa* at 1000 µg/ml gave significantly lower DSI from that treated with crude EtoAC of *E. rugulosa* at 500 µg/ml when compared to that inoculated with *F. oxysporum* f. sp. *lycopersici* NKSC02 as shown in Table 5. Disease immunity to Fusarium wilt in Sida variety showed the highest immunity of 80.95% when treated with crude EtoAC at 1000 µg/ml and followed by that treated with crude EtoAC at 500 µg/ml which showed immunity of 30.09%.

Testing bioformulation of *E. rugulosa* for controlling Fusarium wilt of tomato

The disease severity index (DSI) of Fusarium wilt was the lowest wilt incidence in oil and powder bioformulations (DSI 2 and 1.75), followed by culture filtrate (DSI 2.5), which significantly differed from prochoraz (DSI 4.25) and

Table 5. Effect of crude extracts to induce disease immunity of wilt incidence in tomato var Sida.

Treatments	Plant height (cm)	DSI[1]	Disease immunity[3] (%)
T1 inoculated with pathogen	20.7b[2]	5.25^{a}	-
T2 treated with crude EtoAC of ER at 500 µg/ml	40.75^{a}	3.25^{b}	30.09
T3 treated with crude EtoAC of ER at 1000 µg/ml	41.25^{a}	1.00^{c}	80.95
T4 non inoculated control	41.75^{a}	1.00^{c}	-
CV (%)	9.42	20	

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

Table 6. Testing bioformulations to control Fusarium wilt of tomato *in vivo*.

Treatments	Plant height(cm)	Plant weight(g)	Root weight(g)	Fruit weight(g)	fruits/plant	DSI[1]
Non inoculated control	97.25^{c}	163.25^{b}	6.62^{b}	280.00^{d}	19.50^{d}	1.00^{c}
Inoculated with pathogen	65.75^{f}	73.75^{e}	4.32^{c}	141.25^{f}	10.25^{f}	5.00^{a}
Powder bioformulation	105.25^{b}	168.50^{b}	12.25^{a}	540.75^{b}	29.25^{b}	1.75^{bc}
Oil bioformulation	119.50^{a}[2]	182.25^{a}	13.25^{a}	584.25^{a}	40.50^{a}	2.00^{bc}
Culture filtrate	85.25^{d}	153.75^{c}	6.12^{bc}	430.00^{c}	24.50^{c}	2.50^{b}
Prochoraz	74.00^{e}	137.00^{d}	5.20^{bc}	191.25^{e}	15.00^{e}	4.25^{a}
CV (%)	3.14	1.19	15	3.22	8.71	19.54

[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 to 40%), 4 = plant showed yellowing leaves and wilting (41 to 60%), 5 = plant showed yellowing leaves and wilting (61 to 80%), and 6 = plant showed yellowing leaves and wilting or die (81 to 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 7. Percent increased in plant growth and disease reduction after apply bioformulations.

Treatments	Plant height	Plant weight	Root weight	Fruit weight	Numbers of fruit/plant	DR[2]
POWDER bioformulation	37.25[1]	56.23	64.73	73.87	64.95	58
Oil bioformulation	44.56	61.17	67.39	75.82	74.69	60
culture filtrate	19.35	52.03	29.41	67.15	58.16	50
Prochoraz	11.48	46.16	16.92	26.14	31.66	15

[1]Increased in plant growth parameters = treatment – inoculated control / treatment × 100. [2]Disease reduction (DR) = disease index of inoculated control - disease index of treatment/disease index of inoculated control × 100.

inoculated control (DSI 5.00). The non inoculated control had no wilt incidence. With this, application of oil bioformulation led to reduced wilt incidence of 60%, followed by application of powder bioformulation, culture filtrate and prochoraz which reduced wilt incidence of 58, 50 and 15%, respectively. Based on the result, oil bioformulation was significantly highest in plant height (119.50 cm), followed by powder bioformulation, culture filtrate and prochoraz which were 105.25, 85.25 and 74.00 cm, respectively, when compared to the inoculated control (65.75 cm). Plant weight showed the highest after applying oil bioformulation (182.35 g), followed by powder formulation, culture filtrate and prochoraz, which were 168.50, 153.75 and 137.00 g, respectively when compared to the inoculated control (73.75 g). With this, the root weights of oil and powder bioformulations gave significantly better than culture filtrate and Prochoraz treatments. Oil bioformulation was significantly highest in fruit weight (584.25 g), followed by powder bioformulation (540.75 g), culture filtrate (430 g) and prochoraz (191.35 g) which significantly differed from the inoculated control (280 g). The number of fruits in oil bioformulation application was significantly higher than powder bioformulation, culture filtrate and prochoraz treatments which significantly differed from the inoculated control (Tables 6 and 7).

DISCUSSION

Isolate NKSC02 is confirmed as a virulence that causes wilting of tomato var Sida which was previously reported by Sibounnavong et al. (2010), and this isolate was also confirmed by molecular phylogeny as *F. oxysporum* f. sp. *lycopersici* by Charoenporn et al. (2010) who sequenced the internal transcribed spacer (ITS) region ITS1, 5.8S and ITS2, and a small portion of 18S rDNA and 28S rDNA. *E. rugulosa* could grow very well in mixed media of PDB and CWDB in the range of pH levels from 5 to 8, which is an optimum condition for the growth of *E. rugulosa.* The result was similar to the report of Sibounnavong et al. (2009b) who stated that mixed medium of PDB and CWBD at pH levels 5 to 8 was the optimum condition for the growth of *E. nidulans*.

Crude ethyl acetate from *E. rugulosa* gave the highest inhibition of conidial production of *F. oxysporum* f. sp. *lycopersici* in which ED_{50} value was 138 µg/ml while crude hexane and crude methanol presented their abilities to inhibit conidial production at ED_{50} values of 313 and 1372 µg/ml, respectively. As a result, Sibounnavong et al. (2009a) reported that methanol crude extract from *E. nidulans* gave the highest inhibition of *F. oxysporum* f. sp. *lycopersici*. It is explained that ethyl acetate crude extract from *E. rugulosa* might have different antagonistic substances from methanol crude extract from *E. nidulans* as reported by Moosophon et al. (2006).

Thereafter, separating crude extracts to get pure compound of tajixanthone showed that tajixanthone can actively inhibit *F. oxysporum* f.sp. *lycopersici* NKSC02 at lower concentration than crude extracts which used ED_{50} of 122 µg/ml to inhibit mycelia growth, 54 µg/ml to inhibit macrocondia and 42 µg/ml to inhibit microconidia. Moosophon et al. (2006) isolated pure compounds from *E. nidulans* as epishamixanthone, shamixanthone, emericellin, ergosta-6, 22-diene-3-ol-5, 8-epidioxy-(3β-5α, 22*E*), sterigmatocystin and demethylsterigmatocystin which differed from isolation of pure compounds from *E. rugulosa* as found in five new prenylxanthones, ruguloxanthones A-C, 14-methoxytajixanthone and tajixanthone ethanoate, one novel cyclooctadiene derivative, together with seven known, shamixanthone, tajixanthone, 14-methoxytajixanthone-25-acetate, tajixanthone hydrate, tajixanthone methanoate, isoemericellin and ergosterol (Moosophon et al., 2009). It is indicated that tajixanthone isolated from *E. rugulosa,* firstly reported as a fungal metabolite active against *F. oxysporum* f.sp. *lycopersici,* controls antibiosis, giving it its role of control mechanism.

Apart from the result, crude EtoAC of *E. rugulosa* which contains tajixanthone proved to be a microbial elicitor that induced immunity in tomato. The inoculated tomato seedlings with *F. oxysporum* f.sp. *lycopersici* showed disease immunity to Fusarium wilt in Sida variety of 80.95% where the wilt incidence was much lower than the inoculated control. Hahn (1996) stated that elicitors are molecules that stimulate any of a number of defense responses in plants, such as synthesis of phytoalexins and pathogenesis-related proteins (PR-proteins). Such responses occur after the binding of elicitor molecules to receptors normally located on the plant cell surface, promoting a signal transduction pathway that will lead to the activation of one or more defense mechanisms. The first characterized elicitors were oligosaccharide fragments from fungal cell walls, including oligochitin and oligochitosan (Hahn, 1996).

The biological fungicides have been released and distributed to the growers over a decade. Kaewchai et al. (2009) stated that mycofungicides have been promoted for agricultural use because of their ability to control plant diseases and to increase crop production in an environmental friendly manner. The registered biological fungicide formulated from *C. cupreum* in Thailand could decrease disease incidence of tomato wilt and also increase the yield (Soytong, 1992).

In this study, Fusarium wilt was the lowest wilt incidence in oil and powder bioformulations from *E. rugulosa* which significantly differed from prochoraz and inoculated control. The application of oil bioformulation from *E. rugulosa* could reduce wilt incidence by 60%, followed by application of powder bioformulation and prochoraz which reduced wilt incidence by 58 and 15%, respectively.

This is similar to the work of Charoenporn et al. (2010) who reported that oil bio-agent formulation from other antagonistic fungi of *Chaetomium globosum* and *Chaetomium lucknowense* also showed their biological ability to control tomato wilt. The bio-agent formulations namely N0802, CLT and PC01 gave the highest significant disease reduction of tomato wilt which were 44.68, 36.28 and 41.01%, respectively, followed by prochoraz (21.95%). Charoenporn et al. (2010) stated that all tested bio-agent formulations could significantly increase the yield of tomato when compared to prochoraz and inoculated control. It is concluded that *C. globosum*, *C. lucknowense* and *T. harzianum* which were developed as bio-agent formulations (N0802, CLT and PC01), showed their abilities to control tomato wilt. Based on the result, oil bioformulation from *E. rugulosa* gave significantly better plant parameters in terms of plant height, plant weight, root weight, number of fruits and fruit weight than powder bioformulation and prochoraz when compared to the inoculated control with *F. oxysporum* f. sp *lycopersici*.

This result is similar to the report of Charoenporn et al. (2010) who stated that all tested bio-agent formulations of antagonistic fungi (*C. globosum* and *C. lucknowense*) could significantly reduce tomato wilt caused by *F. oxysporum* f. sp *lycopersici* and increase the yield of tomato when compared to prochoraz and inoculated control. However, bioformulation from *E. rugulosa* in this research finding revealed a good result for controlling wilt

incidence of tomato caused by *F. oxysporum* f. sp *lycopersici.* Soytong et al. (2001) showed that the biological products that 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 reduce infection rate in tomato and those bio-products have been released to the market. It is suggested that this new bioformulation of *E. rugulosa* could be used for further biofungicide to control tomato wilt caused by *F. oxysporum* f.sp. *lycopersici.*

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