

***Fusarium oxysporum* (F221-B) as Biocontrol Agent against Plant Pathogenic Fungi *in Vitro* and in Hydroponics**

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Abstract

Thongkamngam T., Jaenaksorn T. (2017): *Fusarium oxysporum* (F221-B) as biocontrol agent against plant pathogenic fungi *in vitro* and in hydroponics. Plant Protect. Sci., 53: 85–95.

Efficacy of non-pathogenic *Fusarium oxysporum* (F221-B) was assessed as a possible biocontrol agent against fungal pathogens, namely *Curvularia lunata* (C11, C12), *F. semitectum* (F113), *F. oxysporum* f.sp. *lactucae* (F221-R, F442-G), *Rhizoctonia solani* (R11, R12), *Rhizoctonia* sp. (R111, R112, R113) *in vitro*, while F221-B showed a moderate ability to inhibit the mycelial growth of tested fungi about 36–56%. Then, F221-B was further evaluated for its ability of controlling lettuce root rot and wilt caused by F442-G in hydroponics. It was revealed that F221-B reduced disease incidence and severity about 60–80% compared to the inoculated control and significantly promoted the growth of 3 lettuce varieties. Interestingly, using only F221-B gave the significantly highest fresh weight (twice over the healthy control). Conclusively, this study provides an important suggestion for further development of F221-B since it showed the ability of biocontrol agent and plant growth promoting fungus.

Keywords: *Curvularia lunata* (C11, C12); *Fusarium semitectum* (F113); *F. oxysporum* f.sp. *lactucae* (F221-R, F442-G); *Rhizoctonia* sp. (R111, R112, R113); *R. solani* (R11, R12); dual-culture test

Biological control offers a potential alternative control measure in the area of plant diseases and becomes a promising tool to use in agricultural production when it reduces the release of polluting chemical pesticides to the ecosystem (KAUR *et al.* 2010). At present, quite a number of biocontrol agents (BCA) are found to be able to manage the plant diseases effectively as well as they all ecologically sound proof (HOWELL 2003; KAUR *et al.* 2010; KAKVAN *et al.* 2013; CHEN *et al.* 2014; KIM *et al.* 2014; SONG *et al.* 2014). Ideally, the antagonist/BCA must be ecologically fit to survive and function with the particular condition of the ecosystem. Moreover, the antagonist/BCA must be present at an adequate level and be capable of effectively interacting with the pathogen or host plant to provide acceptable disease control.

The wilt and root rot-inducing strains of *Fusarium oxysporum* cause serious losses of many economically important agricultural crops worldwide (BENHAMOU

et al. 1989; HERVAS *et al.* 1998; PAUL *et al.* 1999; KAUR 2003; KAUR *et al.* 2010) including lettuce grown in hydroponics in Thailand (THONGKAMNGAM *et al.* 2012). Effective control methods against the *Fusarium* disease of plants in hydroponics are still limited in terms of an environmentally safe method. Biological control of *Fusarium* wilt and root rot diseases has been reported in numerous crops cultivated in the soil (ALABOUVETTE *et al.* 1993; FRAVEL *et al.* 2003) such as tomato (FUCHS *et al.* 1997, 1999; DUIJFF *et al.* 1998; LARKIN & FRAVEL 1998), cucumber (MANDEEL & BAKER 1991), watermelon (LARKIN *et al.* 1996), spinach (KATSUBE & ALASAKA 1997), basil (MINUTO *et al.* 1997), chickpea (HERVAS *et al.* 1998), and rakkyo (HONDA & KAWAKUB 1998) but not in crops grown in hydroponics especially in the system without substrate.

Non-pathogenic *F. oxysporum* F221-B recovered from roots of lettuce grown in hydroponics (THONGKAMNGAM *et al.* 2012) has shown to significantly

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promote the growth and yield of Cos, Green Oak, Red Oak, and Butterhead lettuce, kale and mung bean in hydroponics (THONGKAMNGAM *et al.* 2013). With the quite promising ability of F221-B, we therefore conducted the research to determine if (1) non-pathogenic F221-B could act *in vitro* as a potent BCA against 10 fungal plant pathogens and (2) F221-B can potentially protect three varieties of hydroponically grown lettuce against the virulent isolate of *F. oxysporum* f.sp. *lactucae* (F442-G) (THONGKAMNGAM *et al.* 2012).

MATERIAL AND METHODS

Fungal isolates. Culture of F221-B was obtained from the previous experiment (THONGKAMNGAM *et al.* 2012, 2013).

The test fungal pathogens were isolated from rice (leaf, seed) and hydroponically grown lettuce (Red Oak, Green Oak) showing disease symptoms from different places (Table 1) using a tissue transplanting technique. *Curvularia lunata* (C11, C12), *F. semitectum* (F113), *R. solani* (R11, R12), *Rhizoctonia* sp. (R111, R112, R113) were obtained accordingly, while the most virulent strains of *F. oxysporum* f.sp. *lactucae* (F221-R, F422-G) were obtained from the previous experiment (THONGKAMNGAM *et al.* 2012). Then, the fungi were transferred onto PDA slants and maintained as stock cultures for this experiment.

***In vitro* assessment of the antagonistic activity of non-pathogenic F221-B against mycelial growth of 10 plant pathogenic fungi by dual-culture test.** F 221-B was determined for its *in vitro* antagonistic activity against ten phytopathogenic fungi by a dual-culture method (DENNIS & WEBSTER 1971) using potato dextrose agar (PDA). A mycelial disc (5 mm) obtained from the peripheral region of the colony 7-day-old cultures of targeted fungal pathogens (C11, C12, F113, F221-R, F422-G, R11, R12, R111, R112, and R113) was placed on PDA at the edge of each plate, 1.5 cm from the periphery. Then, a disc of mycelium of 5 mm in diameter cut from the growing edge of the 7-day-old culture of F221-B was placed on each plate, opposite to the inoculum of the pathogen, 1.5 cm from the periphery. A completely randomised design (CRD) was used with five replicates for each isolate. In the control plate, a sterile agar disc was inoculated on the side opposite to the pathogen. All the inoculated plates were incubated at room temperature ($25 \pm 2^\circ\text{C}$) for 9 days until the

control plate was full. During the incubation period, growth of pathogens was daily measured and the percent growth inhibition (GI) was calculated relative to the control as follows:

$$\text{GI} = ((D1 - D2)/D1) \times 100$$

where: D1 – diameter of pathogen colony in control; D2 – diameter of pathogen colony in treatment

Evaluation of non-pathogenic F221-B for controlling Fusarium root rot of three lettuce varieties grown in hydroponics. F 221-B was further evaluated for its biocontrol efficacy against *Fusarium* root rot and wilt (caused by F422-G) of Butterhead, Cos, and Red Oak lettuce grown in a modified deep flow technique (DFT).

Hydroponic cultivation. Lettuce seeds were germinated in a seedling tray on moist sponge at room temperature. After 7 days, seedlings were moved to a new tray with nutrient solution (EC = 1 mS/cm, pH 5.8–6.2). Then, 14-day-old seedlings were transplanted into mini DFT (plastic container: 17 × 42 × 13 cm) with nutrient solution (EC = 1.6 mS/cm, pH 5.8–6.2) to be ready for being treated according to the treatments.

Experiment. Within each variety of lettuce, four treatments were arranged in CRD with five replications of two plants. Before transplanting the 14-day-old lettuce seedlings into the DFT under outdoor conditions, seedling roots were inoculated with F221-B by dipping into spore suspension (1×10^8 spore/ml and 5 ml per plant). Three days later, the plant roots were inoculated with a spore suspension of the pathogen F422-G (1×10^6 spores/ml and 5 ml per plant), while sterile water was used for healthy control.





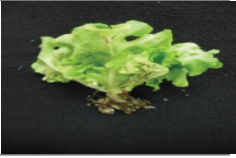





Disease severity was rated up to 6 levels (0 – healthy root, 1 – reddish brown root, 2 – reddish brown root that has become rotten, 3 – rotten root, with slight wilting, 4 – rotten root with severe wilting, 5 – plant that has become dead). Disease incidence (DI) and disease index (DIn) were calculated as follows:

$$\% \text{ DI} = (\text{Number of plants showing infected roots} / \text{Number of total plants}) \times 100$$

$$\% \text{ DIn} = \{[\Sigma(\text{Number of plants showing infected roots} \times \text{disease severity})] / (\text{Number of total plants} \times \text{the highest disease severity})\} \times 100$$

The evaluation was done weekly on DI, DIn, and plant growth (leaf number and size, SPAD value, stem and root fresh weight, stem and root dry weight). Survival of F221-B and pathogen was also monitored

Table 1. Source, host, and isolation description of fungal pathogens used in this study

| No. | Pathogen | Source | Host | Isolation description (symptom) | |
|-----|--|--------------------------|---------------------|---|---|
| 1 | <i>Curvularia lunata</i> C11 | Ratchaburi (Thailand) | rice (seed) | Seeds infected by dirty panicle disease showing black spot, small brown lesion, partial blackening and covered by fungal spores |  |
| 2 | <i>C. lunata</i> C12 | Bangkok (Thailand) | rice (seed) | Undeveloped kernels with severe black spots, brown lesions, partial browning and blackening |  |
| 3 | <i>Fusarium semi-tectum</i> F113 | Ratchaburi (Thailand) | rice (seed) | Undeveloped kernels with brown lesion or black spots covered with whitish spore mass |  |
| 4 | <i>F. oxysporum</i> f.sp. <i>lactucae</i> F221-R | Samutprakarn (Thailand) | lettuce (red oak) | Root rot, yellowing and wilting of basal leaves as well as stunting and plant death. The cortex of the crown and upper root of infected plants became reddish brown and decayed |  |
| 5 | <i>F. oxysporum</i> f.sp. <i>lactucae</i> F422-G | Chonburi (Thailand) | lettuce (green oak) | Disease symptoms included root rot, yellowing and wilting of basal leaves as well as stunting and plant death |  |
| 6 | <i>R. solani</i> R11 | Angthong (Thailand) | rice (leaf) | Grayish leaf lesions (1 × 3 cm) with a red border |  |
| 7 | <i>R. solani</i> R12 | Angthong (Thailand) | rice (leaf) | Black and brown lesions (1 × 5 cm) or stripes on young leaf surrounded with red dots |  |
| 8 | <i>Rhizoctonia</i> sp. R111 | Bangkok (Thailand) | rice (leaf) | Brownish oval lesions (1 × 2 cm) on the leaf sheaths and leaf blades surrounded with yellow area |  |
| 9 | <i>Rhizoctonia</i> sp. R112 | Bangkok (Thailand) | rice (leaf) | Brownish oval lesions (1 × 3 cm) on leaf blades with darkening at the center. |  |
| 10 | <i>Rhizoctonia</i> sp. R113 | Nakornprathom (Thailand) | rice (leaf) | Brownish oval lesions (1 × 1.5 cm) on leaf sheaths at the water level in the paddy field |  |

from a nutrient solution and crop roots at harvest by dilution plates of selective medium.

Data were analysed using analysis of variance (ANOVA). Treatment means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS

In vitro assessment of the antagonistic activity of non-pathogenic F221-B against mycelial growth of ten plant pathogenic fungi using dual-culture

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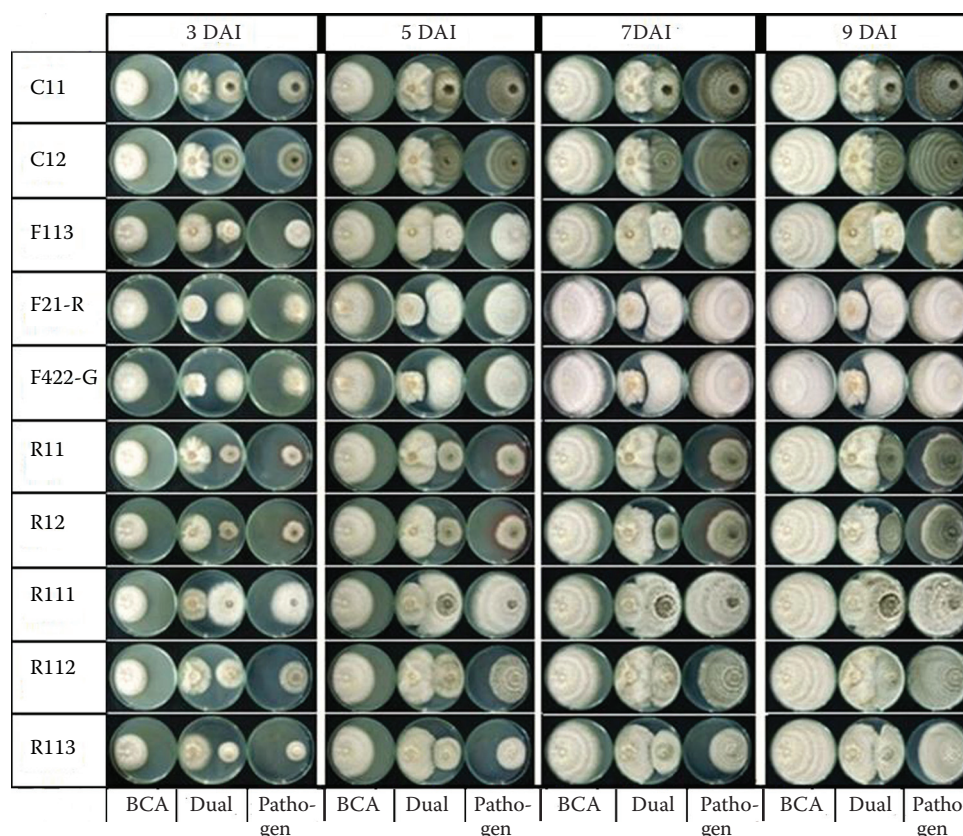
Table 2. Assessment of the antagonistic activity of *F. oxysporum* (F221-B) on colony growth of plant pathogenic fungi using a dual-culture antagonistic test

| Pathogen | Growth Inhibition (%) ¹ | | | | Mechanism ² | | |
|----------|------------------------------------|-------|-------|-------|------------------------|-------|-------|
| | 3 DAI | 5 DAI | 7 DAI | 9 DAI | 3 DAI | 5 DAI | 9 DAI |
| C11 | 5.24 | 19.3 | 30.9 | 42.3 | A | A | A |
| C12 | 6.25 | 17.1 | 24.4 | 36.0 | A | A | A |
| F113 | 10.80 | 25.1 | 29.5 | 42.5 | A | A | A |
| F221-R | 11.90 | 29.0 | 29.7 | 42.0 | A | A | A |
| F422-G | 13.30 | 20.3 | 29.4 | 38.8 | A | A | A |
| R11 | 6.29 | 19.3 | 30.0 | 50.4 | A | A | A |
| R12 | 5.18 | 21.2 | 36.1 | 35.9 | A | A | A |
| R111 | 7.87 | 17.4 | 36.3 | 36.3 | C | C | C |
| R112 | 1.72 | 15.3 | 24.3 | 43.4 | A | A | C |
| R113 | 8.80 | 24.8 | 34.3 | 56.3 | A | A | A |

¹growth inhibition over control (GI) = (D1 – D2)/D1 × 100; DAI – days after inoculation

test. It revealed that non-pathogenic F221-B significantly reduced the colony growth of all tested fungal pathogens at three days after inoculation (DAI) (Table 2). On DAI9, the inhibition percentage for *C. lunata* (C11 and C12) was 42.3 and 36%, respectively; 42.5% for *F. semitectum* (F113); 42 and 38.8% for *F. oxysporum* f.sp. *lactucae* (F221-R and F422-G); 50.4 and 35.9% for *R. solani* (R11 and R12); 36.3, 43.4, and 56.3% for *Rhizoctonia* spp. (R111,

R112 and R113). Considering the above-mentioned inhibition percentages, F221-B showed a moderate ability to inhibit all fungal pathogens. Among the tested fungi, R113 and R11 were the most susceptible and revealed the highest percent inhibition of mycelial growth amounting to 50 and 56%, respectively. Growth inhibition of all tested fungi during *in vitro* interaction with F221-B at DAI 3, 5, 7, and 9 is shown in Figure 1. The main mechanism of F221-B against

Figure 1. Dual-culture antagonistic test of *F. oxysporum* (F221-B) against 10 plant pathogenic fungi at 3, 5, 7, and 9 days after inoculation (DAI)

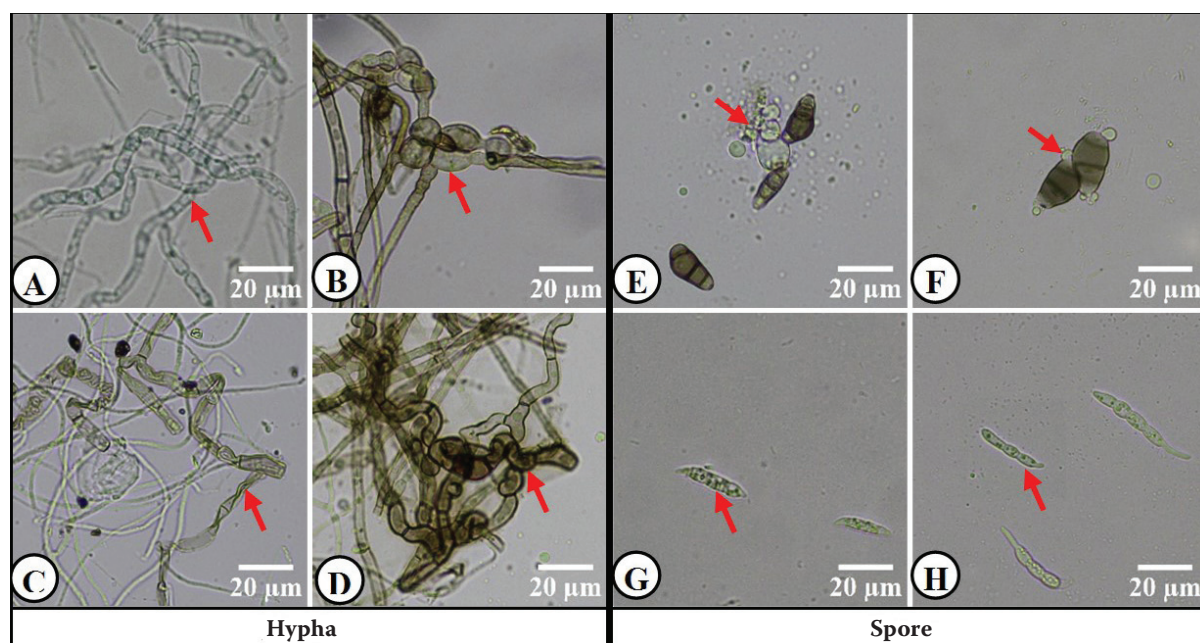


Figure 2. Morphological abnormalities of hyphae and spores of plant pathogenic fungi detected from the interaction zone of dual-culture plate

A = *F. semitectum* (F113), hypha: swollen, with short hyphal segment (yeast-like cell); B = *C. lunata* (C11), hypha: deformities with globose form swelling; C = *R. solani* (R11), hypha: loss of structural integrity showing deformities with shrivelling; D = *C. lunata* (C12), hypha: deformities with swelling and curled; E = *C. lunata* (C11), spore: lysis, loss of cytoplasmic integrity; F = *C. lunata* (C12), spore: cytoplasm leakage out of spore; G = *F. semitectum* (F113), spore: alterations with increased size and coagulation of the cytoplasm; H = *F. oxysporum* f.sp. *lactucae* (F422-G), spore: alterations with increased size and coagulation of the cytoplasm

the tested fungi was attributed to antibiosis and competition. Antibiosis of non-volatile metabolites was more pronounced at the early stage of incubation (DAI 3) and was followed by competition that could be involved in the antagonistic process against both strains of *C. lunata* and *Rhizoctonia* sp. R112. In the F221-B pathogen (R111) combination, only competition was noted from DAI 3 to DAI 9 based on microscopic observations of hyphal interactions, no mycoparasitism was detected. A clear zone of interaction was formed in the combination of F221-B with 6 pathogens, namely R11, R12, R113, F113, F221-R, and F422-G. The presence of the inhibition zone without physical contact between F221-B and the pathogen colony at DAI 5-9 suggested the secretion of diffusible non-volatile inhibitory substances. This was confirmed by the detection of abnormalities of hyphae and spores of the tested fungi (C11, C12, F113, F422-G, and R11) from the interaction zone or nearby area (Figure 2).

Evaluation of non-pathogenic F221-B for controlling *Fusarium* root rot of three lettuce varieties grown in hydroponics. Neither roots of all 3 varieties of lettuces (Butterhead, Cos, and Red

Oak) inoculated with non-pathogenic F221-B alone nor roots of the (uninoculated) control showed any disease symptoms throughout the trial (Figure 3). The roots inoculated with pathogenic F422-G alone were severely rotten with 100% disease incidence (DI) and 69.3–95.7% disease index (DIn) at DAI 28 and Cos was shown to be the most susceptible variety, whereas the treatment with F221-B significantly reduced the percentage of DIn of root rot with the level of control efficacy (over the inoculated control) of 64% for Butterhead, 85.3% for Cos, and 70% for Red Oak (Figure 3). Interestingly, F221-B had a strong antagonistic activity against *Fusarium* root rot especially on Cos lettuce which was the most susceptible variety in our study (Figure 4).

Considering plant growth parameters, results were in line with the disease control efficacy. That is, apart from having the significant control efficacy, the treatment with F221-B (with being challenged with the pathogen) could significantly increase the plant growth (e.g. leaf number and size, SPAD value, fresh and dry weight of stem and root) of tested lettuce over the inoculated control and similarly in relation to the healthy control at DAI 45 (Table 3,

Table 3. Growth of 3 lettuce varieties treated with non-pathogenic *F. oxysporum* (F221-B) and pathogenic *F. oxysporum* (F422-G) in hydroponics

| Lettuce | Treatment | Plant growth parameter | | | | | | | | | | Total yield (g/plant) | | | | | | | | | | |
|-------------|-----------------|------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|-------------------|--------------------------|-------------------|--------------------|--------------------|--------------------|--------------------|------|------|------------|--|--|
| | | leaf number | | | | | leaf size (cm) | | | | | chlorophyll (SPAD value) | | | | | fresh weight | | | dry weight | | |
| | | 24 days | 31 days | 38 days | 45 days | 24 days | 31 days | 38 days | 45 days | 24 days | 31 days | 38 days | 45 days | stem | root | stem | root | stem | root | | | |
| Butter-head | healthy control | 4.2 ^{ab1} | 7.8 ^b | 14.2 ^c | 14.6 ^c | 3.2 ^b | 7.2 ^b | 16.2 ^c | 1.6 ^c | 21.3 ^c | 29.8 ^b | 29.6 ^c | 29.8 ^b | 72.1 ^b | 10.9 ^b | 1.69 ^b | 0.45 ^b | | | | | |
| | F221-B | 4.1 ^a | 9.5 ^a | 18.3 ^a | 23.4 ^a | 4.9 ^a | 8.7 ^a | 19.8 ^a | 28.4 ^a | 31.2 ^a | 33.4 ^a | 34.1 ^a | 35.9 ^a | 115.9 ^a | 19.3 ^a | 2.32 ^a | 0.66 ^a | | | | | |
| | F422-G | 3.8 ^b | 4.3 ^c | 5.8 ^d | 7.6 ^d | 2.5 ^c | 3.4 ^c | 4.2 ^d | 5.25 ^d | 16.4 ^d | 14.9 ^c | 10.4 ^d | 8.5 ^c | 24.5 ^c | 3.2 ^c | 0.78 ^c | 0.12 ^c | | | | | |
| | F221-B+F422-G | 4.8 ^a | 8.4 ^b | 16.9 ^b | 16.1 ^b | 4.5 ^a | 7.6 ^{ab} | 17.5 ^b | 21.2 ^b | 28.4 ^b | 30.8 ^b | 31.7 ^b | 34.8 ^a | 107.7 ^a | 15.1 ^{ab} | 2.03 ^{ab} | 0.54 ^{ab} | | | | | |
| Cos | healthy control | 4.4 ^b | 7.5 ^b | 12.4 ^c | 16.3 ^c | 4.2 ^c | 7.5 ^c | 14.2 ^b | 17.65 ^b | 24.5 ^c | 28.5 ^b | 29.8 ^b | 30.0 ^b | 101.7 ^c | 22.2 ^a | 4.14 ^b | 0.88 ^b | | | | | |
| | F221-B | 5.8 ^a | 9.6 ^a | 18.6 ^a | 26.8 ^a | 6.3 ^a | 15.2 ^a | 21.6 ^a | 27.5 ^a | 31.6 ^a | 31.9 ^a | 32.4 ^a | 35.5 ^a | 192.8 ^a | 26.9 ^a | 5.45 ^a | 1.65 ^a | | | | | |
| | F422-G | 2.7 ^c | 4.2 ^c | 5.5 ^d | 5.9 ^d | 2.6 ^d | 4.5 ^d | 4.9 ^c | 5.12 ^c | 8.4 ^d | 9.2 ^c | 10.4 ^c | 10.5 ^c | 7.2 ^d | 2.5 ^b | 0.3 ^c | 0.1 ^c | | | | | |
| | F221-B+F422-G | 4.5 ^b | 8.5 ^b | 15.4 ^b | 18.3 ^b | 5.1 ^b | 9.8 ^b | 15.3 ^b | 19.2 ^b | 29.6 ^b | 30.8 ^a | 31.5 ^{ab} | 31.2 ^b | 166.5 ^b | 24.2 ^a | 5.13 ^{ab} | 1.0 ^b | | | | | |
| Red Oak | healthy control | 4.1 ^b | 7.5 ^b | 14.4 ^b | 17.5 ^b | 4.2 ^b | 7.8 ^c | 12.2 ^c | 16.5 ^c | 20.4 ^a | 21.5 ^b | 22.8 ^c | 24.5 ^b | 113.3 ^c | 20.5 ^b | 5.76 ^a | 0.78 ^c | | | | | |
| | F221-B | 4.6 ^a | 14.6 ^a | 18.1 ^a | 28.8 ^a | 5.3 ^a | 12.2 ^a | 18.3 ^a | 24.6 ^a | 21.6 ^a | 23.9 ^a | 26.4 ^a | 27.3 ^a | 198.4 ^a | 24.4 ^a | 6.35 ^a | 1.89 ^a | | | | | |
| | F422-G | 3.1 ^c | 3.8 ^c | 4.1 ^d | 4.8 ^c | 3.3 ^c | 4.1 ^d | 4.3 ^d | 4.5 ^d | 10.5 ^b | 11.9 ^c | 14.2 ^d | 10.5 ^c | 37.6 ^d | 4.4 ^c | 1.1 ^b | 0.18 ^d | | | | | |
| | F221-B+F422-G | 4.2 ^{ab} | 6.5 ^b | 13.5 ^c | 18.5 ^b | 4.7 ^{ab} | 8.9 ^b | 13.7 ^b | 18.6 ^b | 20.6 ^a | 21.8 ^b | 24.5 ^b | 25.2 ^b | 151.7 ^b | 21.9 ^{ab} | 6.13 ^a | 1.12 ^b | | | | | |

Means of five replications in a column within each variety of lettuce followed by a common letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $P = 0.05$

Figure 4). Most striking and interesting was the finding that treatment with only F221-B (in the absence of the pathogen) gave the significantly highest fresh weight per plant (115.9, 192.8, and 198.4 g for Butterhead, Cos, and Red Oak, respectively) which were almost twice over the healthy control (Figure 4).

Apart from disease incidence and disease index, the survivals of biological control agent (BCA) and pathogen were also checked at harvest from a nutrient solution and plant roots in terms of colony forming units (CFU/ml). F422-G alone increased its population compared to the original amount while F221-B remained constant throughout the trial. Survivals of F422-G in treatment treated with F221-B were lower than those of the inoculated control (Table 4). F221-B was detected at harvest in the range of 1.2×10^5 – 1.6×10^5 CFU/ml. In the BCA-pathogen co-treated treatment, the amount of F422-G was higher than the original amount but still lower than that in F422-G alone treatment. In addition, the survival of F221-B and F422-G was detected in roots and nutrient solution (Table 4).

DISCUSSION

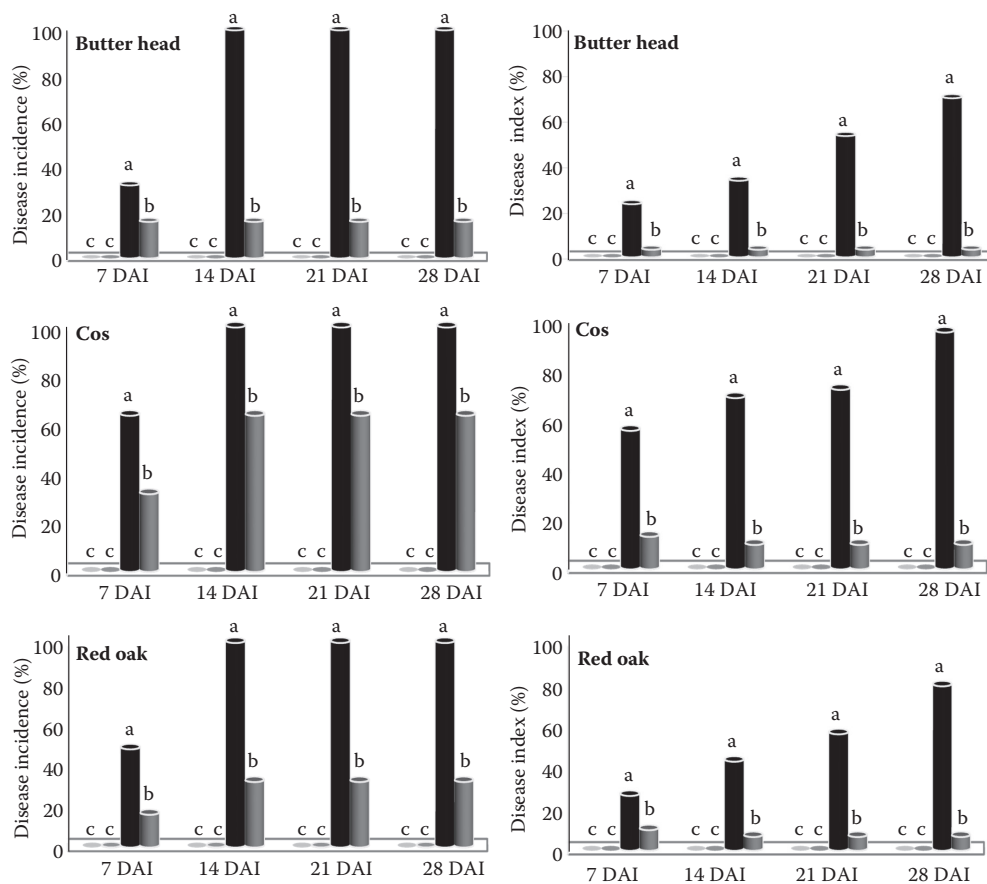
In this study, non-pathogenic F221-B, recovered from roots of hydroponically grown lettuce, was assessed for its possible antagonistic activity against plant pathogenic fungi *in vitro* and in hydroponics. F221-B could considerably inhibit the *in vitro* growth of 10 tested fungi and exerted a direct inhibitory effect e.g. antibiosis and competition. Unlike F221-B in our study, most of the non-pathogenic *F. oxysporum* strains which have been so far reported as promising BCA against fungal pathogens were isolated only from rhizosphere soils and roots of plants grown in suppressive soils (BENHAMOU *et al.* 2002; FRAVEL *et al.* 2003; ABEYSINGHE 2006; RODRIGUEZ *et al.* 2006; PATIL *et al.* 2011) but not from hydroponics. However, these results of the antagonistic activity and mechanisms of F221-B were still in line with those findings. Three non-pathogenic *F. oxysporum* isolates from suppressive soil in Sri Lanka were *in vitro* proved to have an antagonistic potential against pathogenic isolates of *F. oxysporum* f.sp. *radicis-cucumerinum* (ABEYSINGHE 2006), while seven isolates from rhizosphere soils of tomato from Karnataka (India) were reported to have an *in vitro* antagonistic potential against the tomato wilt pathogen (*F. oxysporum* f.sp. *lycopersici*) about 24–40% inhibition with competition mechanism (PATIL *et al.* 2011). *F. oxysporum* against plant pathogens in a dual-culture assay provided the convincing evidence e.g. the non-pathogenic Fo47 exerted a

Table 4. Survival of non-pathogenic *F. oxysporum* (F221-B) and pathogenic *F. oxysporum* (F422-G) in roots (R) and nutrient solution (NS) at the end of the trial

| Lettuce | Treatment | Original amount (spores/ml) | Survival of <i>F. oxysporum</i> (CFU/ml) | | | |
|-------------|---------------|---------------------------------------|--|-------------------|-------------------|-------------------|
| | | | F221-B | | F422-G | |
| | | | R | NS | R | NS |
| Butter head | F221-B | 3×10^5 | 1.6×10^5 | 1.2×10^5 | 0 | 0 |
| | F422-G | 9.5×10^3 | 0 | 0 | 2.1×10^5 | 1.8×10^5 |
| | F221-B+F422-G | $(3 \times 10^5) + (9.5 \times 10^3)$ | 2.2×10^5 | 1.1×10^5 | 1.3×10^4 | 0.3×10^4 |
| Cos | F221-B | 3×10^5 | 1.5×10^5 | 1.4×10^5 | 0 | 0 |
| | F422-G | 9.5×10^3 | 0 | 0 | 2.5×10^5 | 2.2×10^5 |
| | F221-B+F422-G | $(3 \times 10^5) + (9.5 \times 10^3)$ | 2.0×10^5 | 1.7×10^5 | 1.1×10^4 | 1.2×10^4 |
| Red oak | F221-B | 3×10^5 | 1.5×10^5 | 1.6×10^5 | 0 | 0 |
| | F422-G | 9.5×10^3 | 0 | 0 | 2.2×10^4 | 2.1×10^4 |
| | F221-B+F422-G | $(3 \times 10^5) + (9.5 \times 10^3)$ | 1.8×10^5 | 1.5×10^5 | 0.8×10^4 | 1.1×10^4 |

direct inhibitory effect on *Pythium ultimum* through a combination of antibiosis and mycoparasitism (BENHAMOU *et al.* 2002) and the non-pathogenic

S6 had a marked antagonistic capacity against the pathogen *Sclerotinia sclerotiorum* with the responsible metabolite cyclosporine A (RODRIGUEZ *et al.* 2006).

Figure 3. Effect of non-pathogenic *F. oxysporum* (F221-B) on disease development of Fusarium root rot and wilt of lettuce grown in hydroponics

□ – healthy control; ■ – F221-B; ■ – F422-G; ■ – F221-B+F422G; DAI – days after inoculation

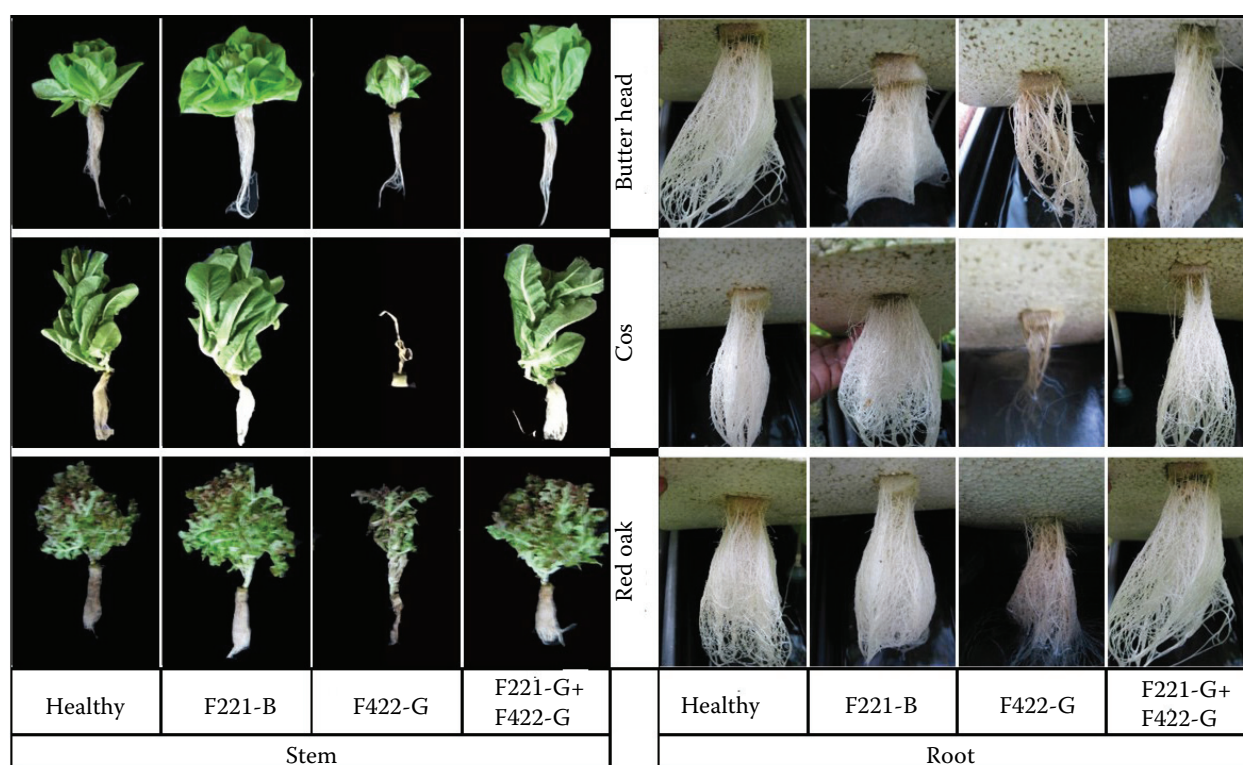


Figure 4. Effect of non-pathogenic *F. oxysporum* (F221-B) and pathogenic *F. oxysporum* (F422-G) on the growth (stem and root) of 3 lettuce varieties in hydroponics at 28 days after inoculation

From our study, the differences in average responses to antagonism by F221-B between the different genera of pathogens imply that genes of the pathogen might be involved in regulating the different levels of antagonism. This confirmed the results of other studies revealing that non-pathogenic isolates from suppressive soils including Fo47 (LEMANCEAU *et al.* 1992, 1993) could differ in their efficacy as well as in their mechanisms (NEL *et al.* 2006).

Another interesting result of this study was that F221-B and pathogenic *F. oxysporum* (F221-R, F422-G) reduced each other's growth when grown together (Figure 1). This fungal antagonism appeared to be mainly associated with antibiosis. The result was in agreement with LEMANCEAU *et al.* (1993), who demonstrated that the same pattern occurred in the co-inoculation experiment between pathogenic *F. oxysporum* (WCS816) and non-pathogenic Fo47.

In the present study, non-pathogenic *F. oxysporum* (F221-B) was investigated as BCA against lettuce root rot and wilt caused by *F. oxysporum* f.sp. *lactucae* F422-G in a deep flow technique. F221-B was found to effectively reduce Fusarium root rot and wilt of 3 lettuce varieties in the range of 64–85.3% as well as to promote their growth and yield. So far, many reports have provided such an evidence of successfully

using many strains of non-pathogenic *F. oxysporum* recovered from either diseased rhizosphere soil or suppressive soil for controlling soil-grown crops (ALABOUVETTE *et al.* 1993; FUCHS *et al.* 1997; DUIJFF *et al.* 1998; FRAVEL *et al.* 2003) but not in hydroponics. Fo47 is the best known and most effective non-pathogenic strain of *F. oxysporum* which was isolated from a soil naturally suppressive to Fusarium wilt of tomato and melon in France (ALABOUVETTE 1990; ALABOUVETTE & COUTEAUDIER 1992; ALABOUVETTE *et al.* 1993, 1996, 1998; LARKIN & FRAVEL 1999) and has been extensively studied for the control of Fusarium wilt disease of several vegetables and flower crops such as tomato (FUCHS *et al.* 1997, 1999; DUIJFF *et al.* 1998), carnation (LEMANCEAU *et al.* 1992, 1993), and flax (DUIJFF *et al.* 1999).

The result gives an important step for F221-B as biocontrol agent to be used in hydroponics. Few reports have revealed the success of using non-pathogenic *F. oxysporum* for controlling plants grown in hydroponics only with the rockwool substrate (GHINI *et al.* 2000; MINUTO *et al.* 2007; HORINOUCHE *et al.* 2008, 2010; NAHALKOVA *et al.* 2008) which is different from this system. Interestingly, F221-B could survive pretty well in such a system (in nutrient solution and plant root). This was in line with

two findings when one demonstrated the ability of non-pathogenic *F. oxysporum* (V2w2 and III4w1) to penetrate the intact host tissue and recolonise the host internally upon inoculation in nutrient solution (PAPARU *et al.* 2006) and the other revealed the capability of non-pathogenic *F. oxysporum* in colonising the lower parts of plantlets such as roots (OLIVAIN & ALABOUVETTE 1999).

The mechanism of action associated with non-pathogenic *F. oxysporum* can be divided into two broad categories: direct antagonism of non-pathogenic strains to the pathogen and indirect antagonism mediated through the host plant such as induced resistance (FUCHS *et al.* 1997). The same non-pathogenic strain can express several modes of action either simultaneously or at different times. This is the case of F221-B in which antibiosis and competition were involved and could be observed in the *in vitro* experiment. However, its mechanism in hydroponics could not be concluded yet.

Regarding this study, F221-B also showed its remarkable ability as plant growth-promoting fungus (PGPF) in addition to its potential in disease control. This confirmed the results of the previous work (THONGKAMNGAM *et al.* 2013) reporting its ability of significantly promoting the growth and yield of Cos lettuce, Green Oak lettuce, Red Oak lettuce, Butterhead lettuce, kale, and mung bean in hydroponics. Other strains of non-pathogenic *Fusarium* were reported as PGPF such as the isolate from rhizosphere soils of tomato in India (PATIL *et al.* 2011), *F. equiseti* GF191 in Japan (HORINOCHI *et al.* 2008, 2010).

CONCLUSION

This study demonstrates that (i) non-pathogenic *F. oxysporum* (F221-B) (THONGKAMNGAM *et al.* 2012, 2013) has the *in vitro* antagonistic ability against 10 plant pathogenic fungi and exerts a direct inhibitory effect namely antibiosis and competition, (ii) F221-B can effectively reduce *Fusarium* root rot and wilt of lettuce especially on Cos, which is the most susceptible variety with control efficacy of 85.3%, and (iii) the newly described F221-B remarkably promotes the growth and yield of three lettuce varieties grown in hydroponics twice over the healthy control especially when it is treated in the absence of the pathogen.

Taken together, the competent non-pathogenic *F. oxysporum* (F221-B) has a great potential as a

promising biocontrol agent and offers a good prospect for integrated management of the root rot and wilt disease of lettuce in hydroponics and probably for other crops as well. However, additional research on F221-B is still needed in several areas including: its colonisation pattern on plant roots, its formulation and applications; repetition of *in vivo* studies with other crops and integration into a production system; risk assessment such as its behaviour and potential impact on hydroponic ecosystem; and probably its genetic improvement.

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