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# Screening fungicides and antagonistic microorganisms for control fruit spot of *Citrus maxima* (Burm.) Merr. cv. Tup Tim Siam pomelo

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**Abstract** The screening of fungicides and antagonistic microorganisms for their ability to control fruit spot disease caused by *Fusarium oxysporum* of *Citrus maxima* (Burm.) Merr. cv. Tup Tim Siam pomelo. The results showed that prochloraz (0.19 mg/ml), thiabendazole (0.60 mg/ml) and thiophanate-methyl (1.05 mg/ml) inhibited mycelial growth 100% and followed with propiconazole (0.25 mg/ml) and fluopyram (0.12 mg/ml) + trifloxstrobin (0.12 mg/ml) inhibited mycelial growth of 85.33 and 83.33%, respectively. The optimal doses to control the causal agent, *Fusarium oxysporum* was prochloraz, thiabendazole and thiophanate-ethyl which were 0.5 mg/ml for 100% mycelial growth inhibition as same as propiconazole at the rate of 1 mg/ml. Moreover, three species of antagonistic microorganisms, *Paenibacillus pabuli* SW01/4, *Trichoderma harzianum* and *Bacillus amyloliquefaciens* KPS46 showed inhibition of mycelial growth of *Fusarium oxysporum in vitro* using poisoned food technique, with inhibition rates of 52.00, 37.44 and 36.00%, respectively.

**Keywords:** Combination, Disease complex, Fruit crops, *Fusarium*

## Introduction

Fruit spot of pomelo caused by *Fusarium* spp. is a major disease problem in *Citrus maxima* (Burm.) Merr. cv. Tup Tim Siam in Nakhon Si Thammarat province, southern Thailand. Pathogens can infect both above-ground and below-ground plant parts, either as primary or secondary pathogens. Usually, economic fruit crops are typically susceptible to *Fusarium* infection in the field condition, and post-harvest, the pathogen can cause vascular wilt, root rot, stem rot, and fruit rot (Zakaria, 2023). *Fusarium* spp. was usually caused wilt symptom on wide range of vegetables, flower plants, fruit crops, and field crops. *Fusarium* spp. has been reported as the causal agent of postharvest disease in several fruit crops including citrus fruit (Schiffmann-Nadel *et al*., 1987), melon (Kim and Kim, 2004), tomato (Chuku *et al*., 2010; Etebu *et al*., 2013; Okey *et al*., 2016),

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banana, papaya, guava (Zakaria *et al*., 2012; Triest and Hendrick, 2016) and pomelo (Amby *et al*., 2015). For fruit rot disease of pomelo, *F. lichenicola* was reported to be the causal agent of fruit rot and it was first reported in Vietnam (Amby *et al*., 2015). Moreover, *F. moniliforme*, *F. solani*, *F. sambucinum*, *F. equiseti* and *F. proliferatum* were isolated from post-harvest decay of citrus fruit in Jeju Island, in South Korea (Hyun *et al*., 2000). *Fusarium* often developed together with *Alternaria* on citrus fruit (Schiffmann-Nadel *et al*., 1987).

The use of fungicides to treat pomelo fruit diseases is the primary control method for reducing yield losses. The fungicides commonly used to control fruit disease are carbendazim, thiabendazole, difenoconazole, pyraclostrobin, trifloxystrobin and fludioxonil (Preecha *et al*., 2018; González-Estrada *et al*., 2020). However, biocontrol is an alternative method that has the advantage of reducing the use of fungicides. Utilizing microorganisms to manage plant diseases can also promote plant nutrition, plant growth, and facilitating interactions between the host plant and other advantageous organisms (Antoun and Prevost, 2006). Antagonistic microoganisms have been reported to control several plant diseases, for example genus *Bacillus*, *Pseudomonas*, *Paenibacillus*, *Streptomyces*, *Chaetomium* and *Trichoderma*.

This research aimed to screen fungicides and antagonistic microorganisms for control of fruit spot of *C. maxima* (Burm.) Merr. cv. Tup Tim Siam pomelo when the causal agent is *Fusarium* spp., *in vitro.*

## Materials and methods

### *Pathogen isolation*

The disease samples were collected from visible canker-spot lesions on the fruit of the Tup Tim Siam pomelo in 2021 September from orchards in Pak Panang District, Nakhon Si Thammarat Province, southern Thailand. The causal agents were isolated using the tissue transplanting method on potato dextrose agar (PDA). The samples were incubated for 1–3 days at room temperature (28–32 °C). The pure culture of *Fusarium* sp. was identified based on morphology.

Pathogeny testing was done using mature fruits (6 months after flowering) of Tup Tim Siam pomelo. Fruits were cleaned by soaking in water and left to dry. The wounding of the fruit was done by punching with sterilized needles for 5 wounds. *Fusarium* sp. was incubated on PDA at room temperature for 5 days. The pathogenicity tested by the peripheral colony was cut with a 0.5 mm sterilized cork borer, and inoculated onto wounded fruits. The control group was neither wounded no inoculated. Fruit spot symptom was determined by measuring lesion size 7 days after inoculation.

### *Screening fungicides for control of Fusarium* sp.

Twenty fungicides were collected from the local agrochemical store for preliminary screening against *Fusarium* sp. using the poison medium technique. The concentrate of fungicides was prepared following the recommended dose. Fungicides were mixed with melted PDA before being poured on to a plate. Pathogenic *Fusarium* sp. cultured on PDA for 5 days was cut with 0.5 cm diameter cock borer and put on the poisoned medium. The mycelial growth of *Fusarium* sp. was measured after incubation at room temperature for 5 days. The high efficacy fungicides used previous testing with varied concentrations to test for the optimal dose using the poison medium technique. The concentration was varied from 0.01 to 0.05, 0.10, 0.50, and 1 mg/ml. The completely randomized design (CRD) was the statistical method of analysis of variance with 21 treatments (20 fungicides and control), each treatment was comprised of 5 replications. A mean comparison experiment was conducted using Duncan's new multiple range test (DMRT).

### *Efficacy of antagonistic microorganisms for control of Fusarium* sp.

Stock culture of antagonistic to *Fusarium* fungus, *T. harzianum* was cultured on PDA for 3 days. The dual culture technique was used in this test. A 0.5 cm disk of pathogen and antagonist for fungal was placed on a PDA disk which was 5 cm apart. Stocks of antagonistic bacterial *B. amyloliquefaciens* KPS46, and *P. pabuli* SW01/4 were cultured in nutrient borth (NB) for 24 hours on a rotary checker at room temperature (28–32 °C). The concentration of antagonistic bacteria was approximately $10^5$ cfu/ml, then streaked for 2 cm on the PDA Petri dish apart from a 0.5 cm disk of pathogen. Inhibition clear zone was evaluated after 3 and 5 days. The data collection method used a formula to calculate the Percent Inhibition of Radial Growth (PIRG).

$$\% \text{ PIRG} = [(R1 - R2) / R1] \times 100$$

Where, R1 corresponds to colony radius of the pathogen in control, and R2 represents the colony radius of the pathogen in treatment (Charoenporn *et al*., 2010). The controls consisted of the pathogen cultured without antagonistic fungi and bacteria. The experimental designs were the Completely Randomized Design with 4 treatments and 5 replications. Duncan's new multiple range test was used with a mean comparison analysis.

## Results

### *Morphology of Fusarium fruit spot disease of pomelo*

Pure culture of *Fusarium* sp. isolated from pomelo fruit spot lesions was study morphological mycelium and conidia. The colony on the PDA was creamy/pale white. The fungus produced both macroconidia and microconidia. Macroconidia were sickle shaped hyaline with three to five septates. Microconidia were small, oval to elongate shaped, hyaline, and single or one septate cell. The pathogen was identified as *F. oxysporum* (Figure 1). When inoculated the hypha tip to mature fruits pomelo cultivar locations Tup Tim Siam, 2–3 mm. brown round spot symptoms appeared on wound inoculation across after 3 days incubation at room temperature. The brown less-sunken spot was 5–7 mm. after inoculation for 7 days (Figure 1).

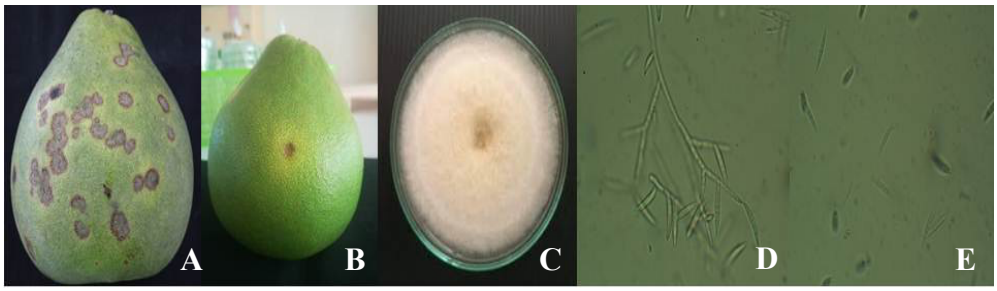


**Figure 1.** Symptoms on fruit spot of Tup Tim Siam pomelo caused by *F. oxysporum* (A), Koch's postulates were confirmed fruit spot disease (B), colony on PDA (C), macroconidia (D), and microconidia (E)

### *Efficacy of fungicides in controlling fruit spot disease on pomelo*

The screening of fungicides for the control the pathogenic *F. oxysporum* causing fruit spot of pomelo showed significantly different results ($P<0.05$). The use of Prochloraz (0.19 mg/ml), thiabendazole (0.60 mg/ml) and thiophanate methyl (1.05 mg/ml) showed high inhibition of *F. oxysporum* mycelium obtained 100%. Whereas propiconazole (0.25 mg/ml), fluopyram (0.12 mg/ml) + trifloxstrobin (0.12 mg/ml), and azoxystrobin (0.06 mg/ml), inhibited mycelial growth of 85.33, 83.33 and 78.22%, respectively ($P<0.05$) (Table 1).

The high efficacy fungicides were trialed for optimal concentration to control *F. oxysporum* using a poison medium. Thiophanate-methyl could completely control, with 100 percentage inhibition, mycelial growth at the lowest concentration of 0.10 mg/ml, while thiabendazole and prochloraz gave complete inhibition at 0.50 mg/ml, and propiconazole at 1.00 mg/ml, respectively. Fluopyram+Trifloxtstrobin, the mixing fungicides were the only ones that did not completely control the pathogen in this trial (Table 2).

**Table 1.** Preliminary screen fungicides collected from the agrochemical stores against *F. oxysporum in vitro* using poison medium technique

| Fungicides | (%) Inhibition of colony |
|---|---|
| Aluminium tris-o-ethyl phosphonate (2.00 mg/ml) | 32.22$^{h1/}$ |
| Azoxystrobin (0.06 mg/ml) | 78.22$^{bc}$ |
| Captan (1.20 mg/ml) | 70.88$^{ef}$ |
| Carbendazim (0.15 mg/ml) | 72.22$^{def}$ |
| Copper hydroxide (1.16 mg/ml) | 22.66$^{i}$ |
| Cyproconazole (0.05 mg/ml) | 77.33$^{cde}$ |
| Difenoconazole (0.11 mg/ml) propiconazole (0.11 mg/ml) | 77.33$^{cde}$ |
| Dimethomorph (0.50 mg/ml) | 73.33$^{def}$ |
| Fluopyram (0.12 mg/ml) + Trifloxtstrobin (0.12 mg/ml) | 83.33$^{bc}$ |
| Hexaconazole (0.05 mg/ml) | 70.00$^{f}$ |
| Mancozeb (1.20 mg/ml) | 43.33$^{g}$ |
| Metalaxyl (0.15 mg/ml) | 28.66$^{h}$ |
| Myclobutanil (0.05 mg/ml) | 76.66$^{cd}$ |
| Prochloraz (0.19 mg/ml) | 100.00$^{a}$ |
| Propiconazole (0.25 mg/ml | 85.33$^{b}$ |
| Thiabendazoles (0.60 mg/ml) | 100.00$^{a}$ |
| Thiophanate-methyl (1.05 mg/ml) | 100.00$^{a}$ |
| Thiram (0.80 mg/ml) | 77.77$^{bc}$ |
| Triadimefon (0.10 mg/ml) | 74.66$^{def}$ |
| Tridemorph (0.19 mg/ml) | 77.77$^{bc}$ |
| Control | 0.00$^{j}$ |

$^{1/}$ = Same letters in the same column indicate that values are not significantly different ($p > 0.05$), mean compared by DMRT.

**Table 2.** Trial for optimal dose of high efficacy fungicides to control *F. oxysporum in vitro*

| Fungicides | % Inhibition of colony | | | | |
|---|---|---|---|---|---|
| | Concentrations (mg/ml) | | | | |
| | **0.01** | **0.05** | **0.10** | **0.50** | **1.00** |
| Fluopyram+Trifloxtstrobin | 86.2$^{c1/}$ | 90.6$^{a}$ | 91.1$^{b}$ | 92.2$^{b}$ | 95.3$^{b}$ |
| Prochloraz | 88.8$^{b}$ | 89.5$^{ab}$ | 89.5$^{c}$ | 100.0$^{a}$ | 100.0$^{a}$ |
| Propiconazole | 79.7$^{d}$ | 88.0$^{b}$ | 88.8$^{c}$ | 90.2$^{b}$ | 100.0$^{a}$ |
| Thiabendazole | 89.3$^{a}$ | 90.2$^{a}$ | 90.4$^{bc}$ | 100.0$^{a}$ | 100.0$^{a}$ |
| Thiophanate-methyl | 89.5$^{a}$ | 90.2$^{a}$ | 100.0$^{a}$ | 100.0$^{a}$ | 100.0$^{a}$ |
| Control | 0.0$^{e}$ | 0.0$^{c}$ | 0.0$^{d}$ | 0.0$^{c}$ | 0.0$^{c}$ |

$^{1/}$ = Same letters in the same column indicate that values are not significantly different ($p > 0.05$), mean compared by DMRT.

***Efficacy of antagonistic microorganisms for control of fruit spot disease of pomelo***

The efficacy of antagonistic test *in vitro*, the result indicated that antagonistic *T. harzianum*, *B. amyloliquefaciens* KPS46, and *P. pabuli* SW01/4 against *F. oxysporum* had a high potential to show antagonism and inhibition of *F. oxysporum.* The antagonistic bacterial *P. pabuli* SW01/4 was the highest control at 52.00% followed by use of *B. amyloliquefaciens* KPS46 was 36.00% inhibition. For the antagonistic fungal, *T. harzianum* showed 37.44% mycelial growth inhibition (Table 3).

**Table 3.** Efficacy of antagonistic *B. amyloliquefaciens* KPS46, *P. pabuli* SW01/4 and *T. harzianum* against *F. oxysporum* testing *in vitro*

| Antagonists | Mycelial growth inhibition (%) |
|---|---|
| *B. amyloliquefaciens* KPS46 | 36.00[c1/] |
| *P. pabuli* SW01/4 | 52.00[a] |
| *T. harzianum* | 37.44[b] |
| Control | 0.00[d] |

[1/] = Same letters in the same column indicate that values are not significantly different ($p > 0.05$), mean compared by DMRT.

## Discussion

*Fusarium* species infecting tropical fruit crops are critical because effective disease management in the field conditions, often depends on identifying the pathogens responsible for Tup Tim Siam disease (Zakaria, 2023). In this study, fruit spot disease of Tup Tim Siam pomelo were identified as *F. oxysporum* (Yeemayee and Dolor, 2021). Fruit spot disease of Tup Tim Siam pomelo cultivation in Pak Panang, Nakhon Si Thammarat, Thailand was a serious disease because of the terrible symptoms in fruit. Fruit appeared with symptoms that made them not marketable. Some species of *Fusarium* associated with diseases of major fruits are *F. oxysporum*, *F. solani*, *F. incarnatum*, *F. proliferatum*, and *F. verticilliodes* (Amby *et al*., 2015; Zakaria, 2023).

In this research we found that fruit spot which grey wide symptom was canker complexed symptom disease with *Fusarium* sp. Even though *Fusarium* sp. normally caused postharvest disease of several fruits including pomelo, in this case it appeared on preharvest fruit on canker lesion. However, *Fusarium* sp. have been reported as part of a complex of diseases with another pathogen such as a complex wilt disease with bacterial wilt and nematode (Bergeson *et al*., 1970; Jorgenson, 1970; Uma Maheswar *et al*., 1997; Castillo *et al*., 1998; Back

*et al*., 2002; Lamichhane and Venturi, 2015; Wanjohi *et al*., 2018; Zakaria, 2023).

Control approach for this pathogen, *F. oxysporum* causing fruit spot was trialed with 20 fungicides collected from various agrochemical stores. The use of prochloraz (0.19 mg/ml), thiabendazole (0.60 mg/ml) and thiophanate-methyl (1.05 mg/ml) were dominant in control efficacy over others at the recommended dose. When variation of concentrates of high control potential from 0.01 to 0.05, 0.1, 0.5 and 1 mg/ml to find optimal dose, the result convinced us that thiophanate-methyl was very low dose for control of this causing agent. It was only 0.1 mg/ml could inhibit 100% mycelial growth when compared to recommendation dose high as 1.05 mg/ml with tenfold. It indicated that the application dose of thiophanate-methyl should be reduced tenfold from recommended dose for control of this pathogen. On the other hand, thiabendazole could control this pathogen at 0.5 mg/ml. It was quite equal with the recommended dose of 0.6 mg/ml. Previous studies informed that *Fusarium* sp. was normally sensitized to benzimidazole (thiabendazole, thiophanate, carbendazim and benomyl) (Hanson *et al*., 1996; Hanks, 1996; Machado *et al*., 2017), but several isolates were resistant to this chemical group (Platt, 1997; Ocamb *et al*., 2007).

In this study, antagonistic bacteria, *P. pabuli* SW01/4 showed high potential for inhibition of mycelium growth of *F. oxysporum. P. pabuli* was the high potential antagonists which has so many articles to confirm their ability (Trinh *et al*., 2018; Yadav *et al*., 2021). *Paenibacillus* species are non-pigmented on media, rod-shaped, motile, and either gram-positive or variable. They can also be facultatively anaerobic or strictly aerobic, and produce ellipsoidal endospores (Trinh *et al*., 2018). They promote plant growth and are biological control agent against many plant pathogens i.e. *Colletotrichum* spp., *Fusarium* spp., *Rhizoctonia* spp. and *Verticillium* spp. (Yadav *et al*., 2021). *P. pabuli* is a plant growth-promoting rhizobacteria (PGPR) (Trinh *et al*., 2018). The effective of PGPR are significantly influenced by changes in the environment, leading to reduced growth challenges, improved plant growth performance, control of environmental pathogens, enhanced nutrient uptake, and induction of plant systemic responses (ISR) (Vejan *et al*., 2016). Moreover, Singh *et al*. (2009) reported that some *Paenibacillus* species exhibit tolerance to commercial fungicides and insecticides. Therefore, this indicates the possibility of using *P. pabuli* SW01/4 in combination with fungicides that were able to reduce the occurrence of fruit spot of pomelo caused by *F. oxysporum* However, the future *P. pabuli* SW01/4 needs to be formulated for convenient practice to implement as alternative control and test in the field conditions.

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