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## The Occurrence and the Approach to Control of Root and Foot Rot of Pummelo (*Citrus maxima* (Burm.)Merr.)var. Tabtimsiam in Nakhorn Si Thammarat Province

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Pummelo (*Citrus maxima* (Burm.)Merr.)var. Tabtimsiam growing in PakPanang, Nakhon Si Thammarat was severely damaged by root and foot rot caused by *Phytophthora parasitica* Dastur. These survey found that disease severity increasing was related to developing stage from young to mature leaf, flowering, young fruit and mature fruit stage. Symptom began at young leaf stage, leaf turned to brown rot cover all leaf and fall down. At fruit stage, symptom of brown rot appeared from young fruit to mature stage, brown rot extended and fruit also fall down. Infectious plant showed stunting, pale and yellow leaf. Main root and stem rotted and gummosis, bark broke out. Severe infect plant, shoot dieback, poor healthy plant with thin canopy appeared, at final stage plant decline most leaf fall down and plant died. Antagonistic bacterial against *P. parasitica* was isolate PNTS06-5, one out of 123 isolates. It inhibited 95.56 % mycelium growth of *P. parasitica*. Fungal antagonist isolate PNTS 02-1, one of 8 isolates inhibited mycelium growth of 75.93 %. Control efficacy against pathogen of antagonistic bacterium and fungus, and fungicides were done *in vitro* and orchard. The result shown that fosetyl-aluminum was the highest efficacy control *P. parasitica* *in vitro* with 100% mycelium growth inhibition. In orchard, fosetyl-aluminum also shows the best control efficacy. It reduced disease severity of 59.80 %. While, antagonistic bacterial isolate PNTS 06-5, antagonistic fungal isolate PNTS02-1 and conventional fungicide (famer application), carbendazim were lower control efficacy of 36.27, 26.47 and 18.62% respectively.

**Keywords:** root and foot rot, antagonist

### Introduction

Pummelo (*Citrus maxima* (Burm.)Merr.) was economic cash fruit of Thailand. Growing area were NakhonPathom, SumutSakhon, Rajaburee, Chinat, Pichit (central of Thailand), PrachinBuree (eastern of Thailand) Chumporn and Nakhon Si Thammarat (southern Thailand). Common name of this citrus plant was pummelo, pomelo, pommelo, pamplemousse, or shaddock. Pummelo is one of the four original citrus species including citron, mandarin, and papeda, while *Citrus grandis* (grapefruit) is a pomelo

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backcross with pummelo × sweet orange (Mabberley, 1997). The commercial cultivars in Thailand were KhaoTongdee (brilliant gold pomelo), , KhaoPaen, KhaoPhuang, KhaoYai, KhaoHom and Bang Khun Non. The cultivar growing in specific area were KhaoTaengkwa, and ThaKhoi, Kao Nam Pueng (white honey pomelo), KhaoGaew and Pattavia (Chomchalow, *et al.*, n.d.). Pummelo var. Tabtimsiamgrowing in PakPanang ,Nakhon Si Thammarat is the new cultivar adapted in PakPanang from indigenous variety at Pattani. The green pear shape which pink red, sweet juice is its dominance over other varieties. The distinctive taste and red juice color rise up the demand and price. This distinctive produce was limited by response of this cultivar on geographic produce only from PakPanang basin growing area. Disease is one of the seriously problem that will reduce yield and produce quality. Greening, tristeza and root and foot rot is the most destructive disease of pummelo. Root rot and foot rot cause fatal disease of citrus and also pummelo. The symptom appear after root system were destroyed 20-30 %. Leaves turned to yellow and some fall, twinge and branch dieback. Plant declined and died at finally. Disease was severe when pummelo was grow on low pH clay soil (Department of Agricultural Extension, Ministry of Agriculture and Cooperative. 2013; Paradornuwat *et al.*, 1984; Sadoodee, 2010).

*Phytophthora parasitica* (Dastur), pathogen of root rot disease was soil fungus which wide host range. Morphological characteristic of this pathogen was hyaline coenocytic hypha, produced round ovoid or pear shape sporangium, sexual oospore from oogonium and antheridium, and pair flagella zoospore. (Gallegly, 2008; Ann *et al.*, 2010; Graham and Timmer, 1994; Manoch, and Chana, C.2012). Control this disease was done by amendment soil with decomposes and suppressing pathogen population by adding antagonistic *Chaetomium cupreum*, *Trichoderma harzianum*, *Bacillus subtilis*, and *B. amyloliquefaciens*. The infected main root and foot should be whittled and coat with metalexyl or fosetylaluminium (Intana *et al.*, 2009; Phung, *et al.*, 2015; Paradornuwat *et al.*, 1999; Soyong, 2010). This research objected to observed disease incidence in the farmer orchard and also trial to developed control methods to reduce root rot and foot rot cause by *P. parasitica*

## Materials and Methods

Survey incident of root and foot rot caused by *P. parasitica* on Pummelo var. Tabtimsiam: was carried out at growing in PakPpanang, Nakhon Si Thammarat. Plant age was category to 3 groups: 1-5, 6-10 years and older than 10 years. Five orchards were sampled and disease symptom was monitor at leaf, twinge, branch, flower, fruit, stem and over soil bare root.

Pathogen, *P. parasitica* was isolated from sample. Infected symptoms collected from farmer orchard in Pakpanung, Nakhon Si Thammarat were isolated by tissue transplanting method on Modified BNPR. For soil sampling from rhizosphere was isolated by soil surface dilution plate. Pure culture was transferred to Carrot Agar (CA), Potato Dextrose Agar (PDA) and V-8 Juice Agar (V-8) for morphological characteristic identification.

Antagonistic fungi and bacteria were isolated from leaves by leaf washing technique and from soil by soil surface dilution plate. Nutrient used to isolate antagonistic bacteria was added cabendazim and metalaxyl (300 µg/ml) and added streptomycin (100 µg/ml) for isolation antagonistic fungi.

Antagonistic screening was done *in vivo*. High potential antagonistic bacterial fungi and bacterial against *P. parasitica* was screened by dual culture technique. Inhibition percentage of antagonists was calculated on mycelium inhibition.

Fungicides were preliminary screened *in vivo*. Fosetyl-aluminium (2.5 mg/ml), mancozeb (1.8 mg/ml), kresoxim-methyl (0.25 mg/ml), copperoxychloride (0.67 mg/ml), carbendazim (0.13 mg/ml), tridemorph (0.11 mg/ml), metalaxyl (0.45 mg/ml), were fungicides which farmer in Pakpanang, Nakhon Si Thammarat application in their orchards. Those were brought to test *in vivo* at recommended dosage as above. Poison media technique was used to screen high potential fungicides to control *P. parasitica*.

Fungicides and antagonists were confirmed screening in greenhouse. *P. parasitica* cultured on Oat meal-sand for 10 days was mixed to sterilized soil medium (soil: sand: manure: coconut dust 1: 1: 1: 3) in 6 inches plastic pot. Seedling of pummelo var. Taptimsiam at 75 days was washed and cut off root tip. The bare root seedling was grown on inoculation soil. Two antagonistic fungal PNTS 01-2 and PNTS 02-1 and three antagonistic bacterial PNTS 06-5, PNPL 01-5, and PNTS 05-2-3 were compared with fosetyl-aluminium, mancozeb, tridemorph and control. Spore suspension of antagonistic fungi cultured on cooking rice was adjusted to  $10^5$  spores/ml. For antagonistic bacterial suspension was adjusted to  $10^6$  cfu. Suspension of fungicides and antagonist were poured to soil medium in plastic pot after transferring pomelo seedling 500 ml/pot. Disease incidence was monitored until 6 months.

Fungicides and antagonists were trialed in farmer orchard. One of antagonistic fungus, bacterium and two fungicides testing in greenhouse at above were trialed in farmer orchard. Three appearing infected symptom at 4 years age pummelo var. Taptimsiam were selected to be tested plant of each treatment. The antagonistic fungus, bacterium and fungicides suspension were prepared as above. The antagonist was added to mix with cow manure and rice bran (1: 4:100; 1: kg), mixed decompose was leave for seven days before applied to rhizosphere of 4 years age infected plants at 5 kg/ plant and

applied 3 time each 30- day interval for 3 month. For fungicide application was sprayed and poured on rhizosphere of infected plants at 20 l/plant each 30-day interval for 3months. Plants healthy recover and diseaseincidence were observed and recorded for six months.

### **Statistical analysis**

Disease incidence, severity and control efficacy were analysis on CRD. Correlation was used to analysis correlating between disease incidence and environmental factors. RBCD was statistical design for trialing at farmer orchard.

### **Results and Discussions**

The survey of root and foot rot on pummelo var. Tabtimsiam was done at growing in PakPanang, Nakhon Si Thammarat. The result showed that *P. parasitica* infected and caused disease on the most part of plant. Disease severity on infected part included shoot/young leaf, mature leaf, flower, young fruit, and mature fruit were 0.83, 1.05, 1.00, 6.17, and 13.83 % respectively. For root infected plant was difficult to observed, but it caused deadly symptom on plant. The first appearance of symptom was observed as similar to nutrient deficiency/ greening/tristeza. Plant declined, leave and fruit fall and died at final. Paradornuwat et al (1984) described that citrus root infected by *P. parasitica* only 20-30% did not showed the appearance symptom, when symptom appear, the plant was severe damage, foot rot, bark loose, leaf and fruit fall. (Paradornuwat *et al*, 1984; Sadoodee, 2010).

Antagonistic bacteria and fungi against *P. parasitica* were screened *in vivo* throughout mycelium inhibition. Five out 123 isolates of bacteria indicated of high control efficacy. The isolation of PNTS06-5, PNPL 01-5, PNTS 05-2-3, PNPL07-4, and PNTS 02-5 showed the high mycelial growth inhibition of 95.56, 89.26, 85.93, 85.19 and 84.44% respectively. Four out 8 isolates of fungi including PNTS 02-1, PNTS 01-2, PNTS 03-2 and PNTS 04-1 were the high mycelial inhibition of 75.93, 67.41, 65.18 and 59.26 % respectively (table 1).

**Table 1.** High control efficacy bacteria and fungi antagonistic isolates against *P. parasitica* *in vivo*.

Bacterial isolate	% inhibition	Fungal isolate	% inhibition
PNTS 02-5	84.44 <sup>c1/</sup>	PNTS 01-2	67.41 <sup>ab1/</sup>
PNTS 06-5	95.56 <sup>a</sup>	PNTS 04-1	59.26 <sup>b</sup>
PNPL 01-5	89.26 <sup>b</sup>	PNTS 03-2	65.18 <sup>b</sup>
PNPL 07-4	85.19 <sup>c</sup>	PNTS 02-1	75.93 <sup>a</sup>
PNTS 05-2-3	85.93 <sup>bc</sup>		

1/= Same letters in the same column indicate that values are not significantly different ( $p > 0.05$ ).

Several fungicides used by farmer were screened for high control efficacy against *P. parasiticain vivo* by poison media technique. Fosetyl-aluminium was the excellent control *P. parasitica* with 100 % inhibited mycelial growth, compared with mancozeb, kresoxim-methyl, copperoxychloride, carbendazim, tridemorph, and metalaxyl of 84.44, 72.22, 64.07, 61.48, 50.00 and 16.67 % respectively (table 2). Metalaxyl was normally high efficacy to control *Phytophthora* spp. Whatever metalaxyl was priority recommend to control *Phytophthora* spp., in this test, the result revealed that its control efficacy was lower than mancozeb. *P. parasitica* resistance to metalaxyl have been report by Cohen and Reuveni (1982) and Sliwka *et al.* (2007). It was possibility that this pathogen should be resistance to metalaxyl *in vivo* test.

**Table 2.** Efficacy control of fungicides to inhibit mycelium growth of *P. parasitica* causing root and foot rot of pummelo (*Citrus maxima* (Burm.) Merr.) var. Tabtimsiam *in vivo*

Fungicide	% inhibition
Fosetyl-aluminium (2.50 mg/ml) <sup>2/</sup>	100.00 <sup>a1/</sup>
Mancozeb (1.80 mg/ml)	72.22 <sup>c</sup>
Kresoxim-methyl (2.5 mg/ml)	64.07 <sup>d</sup>
Copperoxychloride (0.68 mg/ml)	50.00 <sup>e</sup>
Carbendazim (0.13 mg/ml)	16.67 <sup>f</sup>
Tridemorph (0.11 mg/ml)	84.44 <sup>b</sup>
Metalaxyl (0.45 mg/ml)	61.48 <sup>d</sup>
Control	-

1/= Same letters in the same column indicate that values are not significantly different ( $p > 0.05$ ).

2/ = Testing dosage followed product label recommendation

Trial to control root rot of pummelo var. Tabtimsiam seedling caused by *P. parasitica* was carried out in greenhouse. The result showed that fosetyl-aluminium was the highest control efficient of 91.40 %, but it did not distinguish to antagonistic bacteria strains PNTS 06-5, antagonistic fungi strains PNTS 02-1, antagonistic bacteria strains PNTS 05-2-3, and tridemorph with control efficacy 86.96, 80.66, 77.33, 75.84 % respectively (table 3).

**Table 3.** The efficacy of fungicides and antagonists to control root and foot rot of pummelo (*Citrus maxima* (Burm.) Merr.) var. Tabtimsiam seedling caused by *P. parasiticain* greenhouse.

Fungicide and antagonist	Control efficacy
Fosetyl-aluminium (2.50 mg/ml)	91.40 <sup>a1/</sup>
Mancozeb (1.80 mg/ml)	63.62 <sup>cd</sup>
Tridemorph (0.11 mg/ml)	75.84 <sup>abcd</sup>
Fungi strains PNTS 01-2	58.81 <sup>d</sup>
F strains PNTS 02-1	80.66 <sup>abc</sup>
Bacteria strains PNTS 06-5	86.96 <sup>ab</sup>
Bacteria strains PNPL 01-5	67.33 <sup>bcd</sup>
Bacteria strains PNTS 05-2-3	77.33 <sup>abcd</sup>
Control	-

1/= Same letters in the same column indicate that values are not significantly different ( $p > 0.05$ ).

2/ = Testing dosage followed product label recommendation

Fungicides and antagonist that expressed high control efficacy *in vivo* and greenhouse testing were brought to demonstrate at farmer orchard. Control efficacy of fungicides and antagonist were evaluated though the disease severity of test plant. The result showed that disease severity of infected plant treated with fosetyl-aluminium bacterial strains PNTS 06-5 and fungal strains PNTS 02-1 were 34.17, 54.17 and 62.50 % respectively with control efficacy 59.80, 36.27, and 26.47 % respectively. They were higher than carbendazim and untreated control of severity 85.00 and 69.17 % with control efficacy of cabendazim lowest as 18.62% (table4).

**Table 4.** The efficacy of fungicides and antagonists to control root and foot rot of pummelo (*Citrus maxima* (Burm.)Merr.)var. Tabtimsiam caused by *P. parasitica* at farmer orchard.

Fungicide and antagonist	Severity (%)	Control efficacy(%)
Bacterial strains PNTS 06-5	54.17 <sup>b1/</sup>	36.27
Fungal strains PNTS 02-1	62.50 <sup>b</sup>	26.47
Fosetyl-aluminium (2.50 mg/ml)	34.17 <sup>c</sup>	59.80
Mancozeb (1.80 mg/ml)	69.17 <sup>ab</sup>	18.62
Control	85.00 <sup>a</sup>	-

<sup>1/</sup> = Same letters in the same column indicate that values are not significantly different ( $p > 0.05$ ).

2/ = Testing dosage followed product label recommendation

From this research we found that several farmers used the non-effect fungicide (carbendazim) which we indicated the lowest control efficacy *in vivo*, in greenhouse and orchard testing. This trial used cow manure fertilizer to enhance antagonist and plant growth which several reports were supported that manure could prepare nutrient and ecological supporting for antagonist and also for plant growth. (Nesbitt, *et al.*, 1979; Malajczuk and McComb, 1979; Sivapalan *et al.*, 1993, Casale *et al.*, 1995 and Aryantha *et al.*, 2000). Although fosetyl-aluminium and bacterial strains PNTS 06-5 and fungal strains PNTS 02-1 were seemly reduced disease incidence when compared with control, but overall performance of plant was distinguish to healthy plant. So it must take a long time for infected plant to recover back and it spent much more cost without return. From this research, these methods were suitable to protect plant. If plant appeared symptom, we recommend cutting dawn and growing the new one after treating soil with antagonist or fungicide.

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