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## Controlling Powdery Mildew Disease of *Craterostigma pumilum* Hochst. Ornamental Plant

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**Abstract** Powdery mildew was first observed on *Craterostigma pumilum* ornamental plant in November 2014 at Queen Sirikit Botanic Garden, Mae-Rim District, Chiang Mai Province. The fungus was identified as genus *Fibroidium* sp. based on the morphological characteristics of the anamorph using light microscope (LM) and scanning electron microscopy (SEM). The selection of the concentration of environmentally friendly chemical including; aspirin, baking soda, dairy milk (raw cow milk and sterilized milk), were tested for inhibition of spore germination on thin layer of onion. The percentage of spore germination inhibition was gradually increased by increasing the tested concentration compared to the control treatment (water only). The experiment indicated that aspirin (13.38g/20L), baking soda (50g/20L), raw cow milk (10%), sterilized milk (10%) compare with the commercial fungicide carbendazim (10cc/20L) and sulfur (30g/20L). Then, the next experiments on *C. pumilum* were conducted under greenhouse condition during 2015 growing seasons for management of powdery mildew. In these trials, the results showed that revealed spray commercial fungicide sulfur was the superior treatment in this regard, being 85.71% efficiency followed by spraying of raw cow milk (77.38% efficiency). Then, carbendazim, baking soda and sterilized milk (71.42% efficiency). Meanwhile, aspirin was the lowest efficient in reducing the severity of the disease being 60.71% efficiency. Control treatment recorded 70% disease severity.

**Keywords:** *Craterostigma pumilum* Hochst, Controlling powdery mildew, management plant disease, powdery mildew, *Podosphaeria* sp.

### Introduction

*Craterostigma* Hochst. is a herbaceous genus distributed in tropical and Southern Africa, Southern Arabian Peninsula, and India (Blundell, 1987; Fischer, 2006). In the Arabian Peninsula, Wood (1997) described two species from northern Yemen, namely: *Craterostigma pumilum* Hochst. and *C. plantagineum* Hochst., while in Saudi Arabia, one species only was described *C. pumilum* Hochst. (Collenette, 1985, 1999; Chaudhary, 2001). In the foreign country Charuvi *et al.* (2015) using *C. pumilum* as a model

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plant to investigate the alterations in the supramolecular organization of photosynthetic protein complexes. Nowadays, *C. pumilum* entered to Thailand as an ornamental plant during January 2014 at a greenhouse in Queen Sirikit Botanic Garden, Mae-Rim District, Chiang Mai Province.

Powdery mildew was first observed on *C. pumilum* during November 2014 at the end of the winter rain season. Once *C. pumilum* leaves are infected with powdery mildew it is difficult to control, if left unchecked the crop can be entirely destroyed. This is the most serious disease attacked on *C. pumilum* causes considerable losses in yields in the greenhouse.

Chemical control is highly recommended that the powdery mildew is an aggressive and destructive disease and satisfactory control without the use of fungicides is unlikely. The role of fungicides in reducing the disease is well known (McGrath, 2001; 2004). The chemical fungicides have been used as the main strategy for control of powdery mildew disease and subsequently increases yield production (Abdel-Moneim *et al.*, 1980; Keinath and DuBose, 2004; Wolf and Verreet, 2008). Unfortunately, the current and indiscriminate use of the fungicides posed a serious threat to human health, environment and production of fungicide resistant pathogen strains (McGrath, 1991; Garcia, 1993 and Durmusoglu *et al.*, 1997 and Fernandez-Aparicio *et al.*, 2009). The great efforts by agro-scientists are carried out to search and development of nontoxic alternative to chemical fungicides would be useful in reducing the undesirable effects to uses for management plant disease, for example aspirin, baking soda and raw cow milk.

Therefore, the main purpose of this study was to identify a pathogen of powdery mildew on *C. pumilum* and control powdery mildew by environmentally friendly chemical.

## **Material and methods**

### ***Sample sources***

Powdery mildew fungi specimens were collected at Queen Sirikit Botanic Garden, Mae-Rim District, Chiang Mai Province, Thailand from 2015. The herbarium specimen was deposited at the mycological herbarium in Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Thailand.

### ***Morphological observation***

#### **Light microscopy**

Fungal colonies on fresh specimens were stripped off by adhesive tape, mounted in distilled water and examined by standard light microscopy with 40X objective phase contrast lenses. Morphological characteristics

were measured in 30 replicates for each structure: size and shape of conidia, conidiophore; position of the basal septum; shape and position of hyphal appressoria and presence or absence of fibrosin bodies (To-anun *et al.*, 2005). Observation of conidial germ tube was carried out using the method of Hirata (1942).

#### **Scanning electron microscopy (SEM)**

Suitable areas were selected using a dissecting microscope. Leaf areas containing powdery growth were cut and placed on double-sided cello tape then sputter coated with gold using Fine coat ion sputter. Gold-coated samples were then placed on aluminium stubs in a JEOL JSM 6360 SEM (JEOL 5410, Tokyo, Japan). Characters examined were the surface pattern of conidia.

#### ***Pathogenicity tests***

Pathogenicity was confirmed by dusting conidia on healthy plants of *C. pumilum* and non-inoculated plants served as controls. A sterile brush was used to transfer conidia from the affected leaves to fully expanded leaves of healthy plants. A plastic bag was placed around each plant for a week and then removed. Non inoculated controls were stroked with a sterile brush, placed in a plastic bag and kept separate in the greenhouse.

#### ***Laboratory experiments***

##### **Effect of the environmentally friendly chemical on spore germination of powdery mildew**

According to the methods adopted by Nair and Ellingboe (1962), powdery mildew spores were harvested from only young leaves of *C. pumilum*. The new conidia were spread on thin layer of onion (size: 1×1 cm<sup>2</sup>). Carrying 1 ml. of each environmentally friendly chemical at different concentrations and carrying 1 ml of sterile distilled water served as control. The inoculated cell layer of onion was floated on distilled water in a petri dish at room temperature for 24 hour. Five replicates were used for each treatment, examined with a light microscope to observe conidial germ tube. The percentages of germinated spores were estimated according to the following formula:

$$\text{Percentage of germination} = \frac{\text{No. of germinated spores}}{\text{Total number of spores}} \times 100$$

$$\text{Percentage of germination inhibition} = 100 - \text{Percentage of germination}$$

## Greenhouse experiments

### Assessment of Powdery Mildew Severity on Leaves

Naturally infected leaves by *C. pumilum* powdery mildew were assessed after 6 weeks. Twenty-four leaves were randomly selected from six pots, examined to assess the severity of the disease depending on the devised scale 0–5 adopted by Townsend and Heuberger (1943) and Biswas *et al.* (1992) (Figure 1).

Where:

0 = No powdery mildew colonies observed,

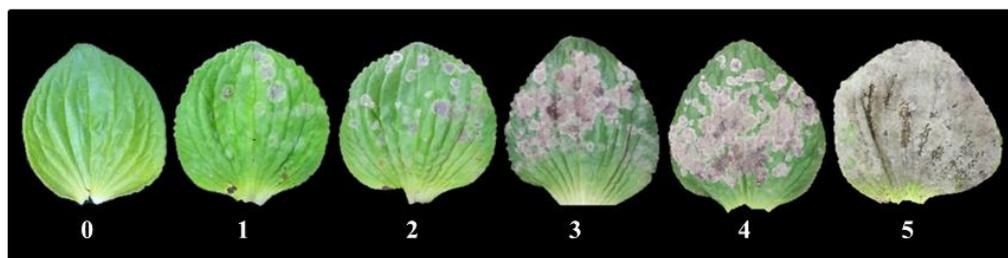
1 = 1–20% of the leaf area infected,

2 = 21–40% of the leaf area infected,

3 = 41–60% of the leaf area infected,

4 = 61–80% of the leaf area infected,

5 = 81–100% of the leaf area infected.



**Figure 1.** Diagrammatic scale of *C. pumilum* showing increasing percentages for assessment of powdery mildew severity

The severity of the disease was calculated using the following formula:

$$\text{Disease severity \%} = \frac{\sum (n \times v)}{5N} \times 100$$

Where:

n = Number of the infected leaves in each category.

v = Numerical values of each category.

N = Total number of the examined leaves.

The efficiency of the treatments were calculated according to the following formula:

$$\% \text{ Efficiency} = \frac{\% \text{ Infection in the control} - \% \text{ Infection in the treatment}}{\% \text{ Infection in the control}} \times 100$$

### **Effect of environmentally friendly chemical on *C. pumilum* powdery mildew severity**

Greenhouse experiments were carried out during February – April 2016 at Queen Sirikit Botanic Garden, Mae-Rim District, Chiang Mai Province, Thailand. The plants were left to the natural infection by powdery mildew and distributed in a complete randomized block design (RCBD). Apply spray treatments every 7 days. Where six pods were used as a replicate and twenty four leaves replicates were used for each treatment.

#### ***Statistical Analysis***

Data were statistically analyzed using the standard procedures for complete randomized block designs. The averages were compared at 5% level using least significant differences (LSD).

### **Results**

#### ***Symptoms***

On *C. pumilum*, white, powdery colonies initially appeared as patches on the upper surfaces of the lower leaves. The colonies subsequently became thicker, expanded, and merged to cover entire leaves; at the same time, new colonies spread successively to the upper leaves (Figure 2).



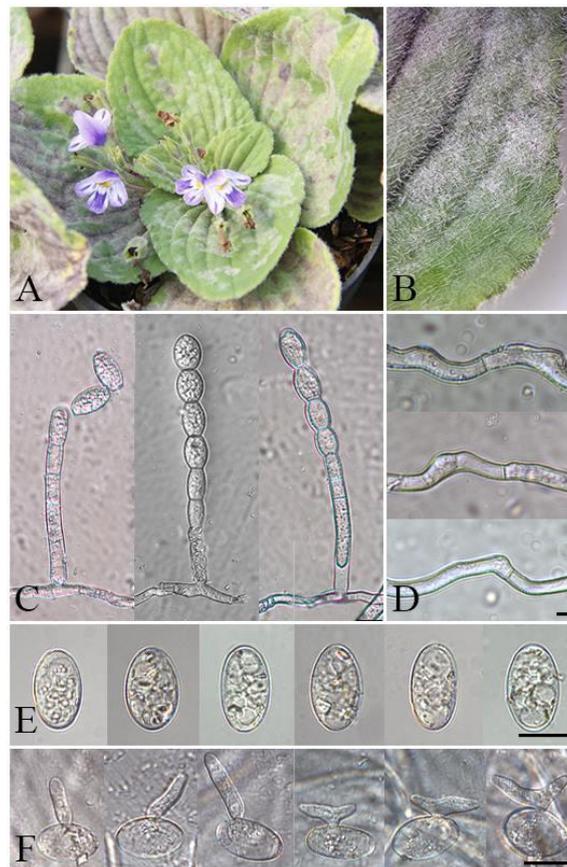
**Figure 2.** The symptoms of powdery mildew disease on *C. pumilum* leaves

#### ***Morphological observation***

##### **Light microscopy**

The powdery mildew fungi having present only anamorphic states were identified using the general keys to species as described in Key to Genera of the Powdery Mildew Fungi (Erysiphaceae) by To-anun and Takamatsu (2005).

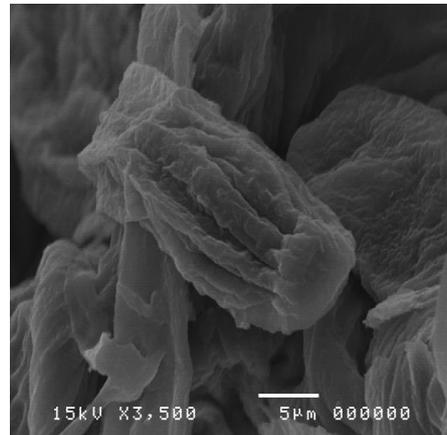
The observations revealed the presence of ectophytic mycelium, white, dense patches or covering the entire lower leaves surface; hyphal appressoria, indistinct or nipple-shaped; Conidiophores were straight to slightly curved measured (11.25)13.75–20.00 × (77.50–)107.50–190.00(–222.50) μm (average 16.25 × 151.63 μm), containing a mother cell forming conidia in chains, (5.00–)7.50–15.00(–20.00) × (30–)45.00–70.00(–75.00) μm (average 11.17 × 58.58 μm); foot cell usually straight, (10.00–13.75(–17.50) × (28.75–)35.00–67.50(–100.00) μm (average 11.75 × 53.80 μm); conidia were hyaline, ellipsoid to ovoid containing conspicuous fibrosin bodies, 17.50–21.25(–22.50) × (28.75–)30.00–36.25(–37.50) μm (average 19.71 × 33.33 μm); conidial germination formed fuliginea-type or pannosa-type; present on the lateral side of the conidia. (Figure 3). A perfect stage (chasmothecium) was not found. This is the first report of powdery mildew on *C. pumilum* caused by *Fibroidium* sp. (*≈podosphaera* sp.) in Thailand.



**Figure 3.** Powdery mildew infections of *C. pumilum* by *Fibroidium* sp. (A) a severe infections on the plants, (B) Close-up view of infections on leaves, (C) conidiophores, (D) appressoria, (E) conidia with fibrosin bodies, (F) conidia with germ tubes of the fuliginea-type and pannosa-type. – Bars 10 μm.

### Scanning electron microscopy (SEM)

Observation on the surface of on conidia of fungal using scanning electron microscope revealed that the outer wall surface was sinuous outer wall and fibrillar septa (Figure 4).



**Figure 4.** Wrinkling pattern on conidia of *Fibroidium* sp. on *C. pumilum*. Bar = 5 µm.

### Pathogenicity test

The pathogen signs were observed only in inoculated plants at 10 days after inoculation. Thin, white colonies were observed on leaves of with powdery mildew, whereas control plants remained healthy (Figure 5).



**Figure 5.** Results of pathogenicity tests after 7 days. (A) inoculated, (B) control

### Laboratory experiments

#### Effect of some organic based products on spore germination of powdery mildew

Data presented in Table 1 revealed that all the environmentally friendly chemicals tested were highly effective in reducing spore germination. The percentage of spore germination inhibition was gradually increased by increasing the tested concentration compared to the control treatment (water only). Treatments with 2 concentrations (8.79 & 13.38 g/20L) of aspirin showed the inhibition (77.96–85.95%) in conidial germination. Treatments with 2 concentrations (50 & 100 g/20L) of baking soda showed the inhibition (81.26–83.19%) in conidial germination. Treatments with 3 concentrations (10, 50 and 100%) of raw cow milk showed the inhibition (85.12–86.22%) in conidial germination. While, treatments with 2 concentrations (5 & 10 cc/20L) of carbendazim showed the inhibition (81.26–90.08%) and treatments with 2 concentrations (15 & 30 g/20L) of sulfur showed the inhibition (87.32–93.66%).

However, the analysis of statistics and taking into consideration the appropriate value, found that the appropriate concentration to control the disease in greenhouse conditions were aspirin (13.38g/20L), baking soda (50g/20L), raw cow milk (10%), sterilized milk (10%), compare with the commercial fungicide carbendazim (10cc/20L) and Sulfur (30g/20L). Use for the next experiment.

**Table 1.** Effect of environmentally friendly chemicals on spore germination of powdery mildew, 24 hour after incubation at room temperature.

Treatments	Percentage of spore germination inhibition <sup>1</sup>
Aspirin (8.79g./20L)	77.96h <sup>2</sup>
Aspirin (13.38g./20L)	85.95cdef
Baking soda (50g./20L)	81.26g
Baking soda (100g./20L)	83.19fg
Raw cow milk (10%)	85.12def
Raw cow milk (50%)	85.39def
Raw cow milk (100%)	86.22cde
Sterilized milk (10%)	83.47efg
Sterilized milk (50%)	84.29ef
Sterilized milk (100%)	88.43bc
Carbendazim* (5cc./20L)	81.26g
Carbendazim* (10cc./20L)	90.08b
Sulfur** (15g./20L)	87.32bcd
Sulfur** (30g./20L)	93.66a
Control	–
LSD (0.05)	2.81
CV (%)	2.60

\*Systemic fungicide (positive control); \*\* Contact fungicide (positive control)

<sup>1</sup>The average was calculated using data from five replications.

<sup>2</sup>Values in the same column with different superscripts significantly differed at P=0.05.

*Greenhouse experiments***Effect of environmentally friendly chemical on *C. pumilum* powdery mildew severity**

Data presented in Table 2 show the effect of spraying of *C. pumilum* on management of the natural infection by powdery mildew during 2016.

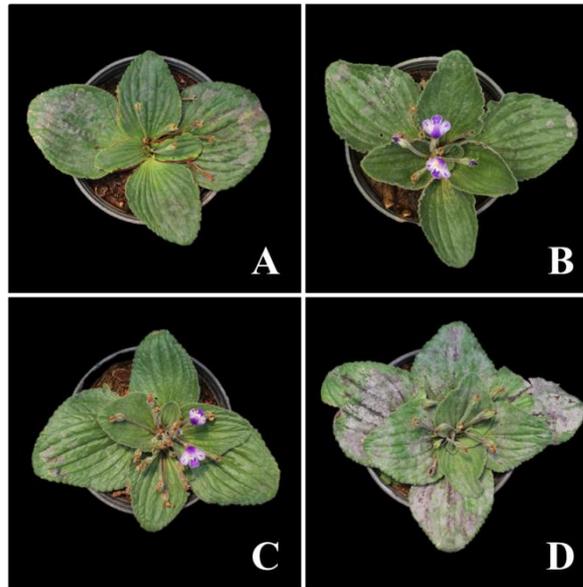
Table 2 showed that spraying of any of the aforementioned tested treatments significantly reduced the severity of powdery mildew compared with control treatment. In addition, sulfur was the superior treatment in this regard, being 85.71% efficiency followed by spraying of raw cow milk (77.38% efficiency). Then, baking soda, carbendazim and sterilized milk (71.42% efficiency). Meanwhile, Aspirin was the lowest efficient in reducing the severity of the disease being 60.71% efficiency. Control treatment recorded 70% disease severity.

**Table 2.** Effect of spraying of *C. pumilum* powdery mildew with environmentally friendly chemical on the severity of powdery mildew.

Treatments	Disease Severity %	Efficiency %
Aspirin	27.50b	60.71c
Baking soda	20.00bc	71.42b
Raw cow milk	15.83c	77.38b
Sterilized milk	20.00bc	71.42b
Carbendazim	20.00bc	71.42b
Sulfur	10.00c	85.71a
Control	70.00a	–
LSD (0.05)	10.41	8.22
CV (%)	33.72	9.47

<sup>1</sup>The average was calculated using data from twenty–four replications.

<sup>2</sup>Values in the same column with different superscripts significantly differed at P=0.05.



**Figure 6.** Effect of foliar spraying environmentally friendly chemicals on severity of powdery mildew on *C. pumilum* after 6 weeks (A) aspirin, (B) raw cow milk, (C) sulfur, (D) control

## Discussion

Several powdery mildew species have been reported on ornamental plants. However, there is no record and have been reported of powdery mildew on *C. pumilum* in the world. This is the first report of powdery mildew on *C. pumilum* in Thailand and the world. The fungus was identified as genus *Fibroidium* sp. based on the morphological characteristics of the anamorph using light microscope and scanning electron microscopy. The characterized demonstrated that the conidia in chains, fibrosin bodies and indistinct appressoria, which indicated *Podosphaera* and excluded the possibility of *Erysiphe*, *Golovinomyces* and *Leveillula* (Braun *et al.*, 2002). Germ tubes were present on the lateral side of the conidia and were branched (*Fibroidium* subtype *brevitubus*), which further supported our hypothesis of this pathogen belonging to *Podosphaera* sp. (Cook and Braun 2009). A perfect stage (chasmothecium) was not found. Smooth wrinkles pattern were evident in SEM studies (Cook *et al.*, 1997) revealed that the outer wall surface was sinuous outer wall and fibrillar septa.

The pathogenicity test was confirmed by inoculated plants developed symptoms after 7–10 days whereas control plants remained healthy. Similar results were obtained by Pankaj *et al.* (2010) reported, inoculated plants developed symptoms after 10 days test by *Podosphaera* sp. on *Coreopsis* sp. in India.

In this research finding, environmentally friendly chemical were tested for inhibition of spore germination on thin layer of onion. The percentage of spore germination inhibition was gradually increased by increasing the tested concentration compared to the control treatment (water only). The experiment indicated that aspirin (13.38g/20L), baking soda (50g/20L), raw cow milk (10%), sterilized milk (10%) compare with the commercial fungicide carbendazim (10cc/20L) and sulfur (30g/20L).

The efficiency on management of *C. pumilum* powdery mildew under greenhouse conditions during 2015 at Queen Sirikit Botanic Garden. The obtained data showed that spraying of *C. pumilum* with any of the tested treatments significantly reduced the severity of powdery mildew with control treatment. The results of revealed that spray raw cow milk 10% was the superior treatment in this regard, being 77.38% efficiency. This was similar to the report of Crisp *et al.* (2006), who reported that used eight spraying treatments with solution of milk 1:10 or 45 gr/l of whey powder or combination of alternating spraying every 10–14 days with potassium bicarbonate with oil and whey were efficient as much as sulphur. Sudisha *et al.* (2011) used cow whole milk (CWM) at 5, 10, 20 and 30% to examine the preventive control against downy mildew disease [*Sclerospora graminicola* (Sacc.) J. Schröt.] and observed the highest disease protection at 10% (100mL L<sup>-1</sup>). In addition, Bettiol (1999) has reported that foliar sprays of aqueous dilutions of fresh, non-pasteurized cow's milk can be quite effective in controlling *Podosphaera xanthii* on zucchini (*Cucurbita pepo* cv. *caserta*) in the greenhouse. Weekly foliar sprays using an aqueous solution with 40% milk by volume were observed to reduce disease severity by 85–90%. Milk is rather heterogeneous suspension of oil (butterfat), protein (cassein), sugar (lactose) and a multitude of possibly bioactive trace ingredients, including minerals, enzymes and vitamins. Bettiol (1999) provides a review of the possible modes of action for milk-based sprays. These include an increase in the pH of the leaf surface (Ziv and Zitter, 1992). However, the use of milk sprays may present some difficulties. Bettiol (1999) reported problems with a sooty mould at high milk concentrations on zucchini in the greenhouse.

Other applications, baking soda has effective similar to carbendazim and sterilized milk. It's used to control powdery mildew because their proposed advantages include low cost, favorable safety profile for humans and the environment and low mammalian toxicity (Reuveni and Reuveni, 1995a; Olivier *et al.*, 1999). Various bicarbonate salts are suggested as a good option to control powdery mildew. Efficacy of bicarbonate against powdery mildew has been proven in various crops, including grape powdery mildew (*Uncinula necator*), Sawant and Sawant, 2008; cucumber, Homma *et al.*, 1981, Ziv and Zitter, 1992; rose powdery mildew (*Spaerotheca pannosa*), Horst *et al.*, 1992; apple powdery mildew (*Podosphaera leucotricha*), Beresford *et al.*, 1996. The use of

aspirin has been reported to induce resistance in plant. But, not report in controlling powdery mildew. In 1979, White showed that the exogenous application of salicylic acid (SA) and certain other benzoic acid derivatives induces both resistance to TMV and the accumulation of PR-proteins. Lopez-Lopez *et al.* (1995) reported the increased resistance in potato tubers against *Erwinia carotovora* ssp. *carotovora* was observed when tuers were dipped in acetylsalicylic acid (ASA). In the experiments, we found that aspirin was the lowest efficient in reducing the severity of the disease compared to other treatments. Moreover, application of aspirin rate 10g/20L was clearly phytotoxic cause's leaf burning. This present study suggests the potential of developing environmentally friendly chemicals for control of *C. pumilum* powdery mildew disease.

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