



## Efficacy of *Ampelomyces* spp. against powdery mildew disease of rose caused by *Podosphaera pannosa*

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### Abstract

The fungus *Ampelomyces* spp., a hyperparasite of powdery mildews, was isolated from flowering plants *Zinnia elegans*, *Ageratum conyzoides*, *Hydrangea* sp., and *Dahlia* spp. The morphological characteristics were observed using light and scanning electron microscopes. Pycnidia variable in shape (ovoid and ellipsoid) and conidia measuring 10.5 to 13.5  $\mu\text{m}$  in length  $\times$  2.5 to 3.0  $\mu\text{m}$  in width were detected. Four isolates of *Ampelomyces* spp. were obtained, AMP1-Ze, AMP2-Ac, AMP3-Hg and AMP4-DI, to study their ability to inhibit conidial germination on onion cell tissue of the fungus *Podosphaera pannosa* which causes rose powdery mildew. The biocompounds produced by these mycoparasites had LC<sub>50</sub> values between 91.20-190.55 ppm. The most effective biocompound was produced by the *Ampelomyces*–from *Ageratum conyzoides* (AMP2-Ac) and it was compared to a conventional fungicide (carbendazim 10cc/20L) and control (non-treated). This biocompound reduced the severity of powdery mildew disease by 45.33% in greenhouse experiments.

**Key words** – Antagonistic fungi – biological control – hyperparasite – *Podosphaera pannosa*

### Introduction

Powdery mildew of rose caused by *Podosphaera pannosa*, is an obligate biotrophic parasite that results in epidemics in greenhouses and landscapes at temperatures of 21-25°C and low humidity (Paulitz & Bélanger 2001). This disease is severe on susceptible rose cultivars in Chiang Mai province, Thailand. The conidiophores of *P. pannosa* produce apical conidia which contain fibrosin bodies (Cook & Braun 2009). Conidia and conidiophores can be spread by wind, and possibly by insects. Infections appear on young aerial parts of plants covering them with whitish colonies. Infected plants become distorted and have reduced production (Carlo et al. 1997). Using fungicides for control of rose powdery mildew disease has increased the level of applied chemicals and production costs. In addition, chemical fungicides can be phytotoxic and disrupt the antagonistic microbial activity of non-target microorganisms.

Alternative methods for control of powdery mildew including the use of biological control agents (BCAs) are promising. Mycoparasites belonging to the genus *Ampelomyces* have been found on a wide host range of more than 64 species of powdery mildew (Dario et al. 2009). They have been most effective against powdery mildew fungi in the Erysiphaceae such as *Erysiphe cichoracearum*, *Uncinula necator*, *Podosphaera leucotricha*, and *P. pannosa* which causes rose powdery mildew (Puzanova 1991). The infection process of powdery mildew by *Ampelomyces* conidia and hyphae occurs by germ tubes which penetrate into host cells. Pycnidia are formed within hyphae and vary in shape depending on host fungal structure. Moreover, *Ampelomyces* spp. produce toxic metabolites and can be mass produced in artificial liquid media. Extracts of *Ampelomyces* spp. isolated from *Urospermum picroides* contained a new pyrone and sulfated anthraquinones. However, fungal growth rate was slow a few mm in solid culture and not feasible for commercial mass production in this manner (Dario et al. 2017). Although conidia of *A. quisqualis* isolated from *Podosphaera leucotricha* can germinate on water agarose medium, conidia germination decreased as conidial concentration increased at 8, 10, 20 and 34x10<sup>6</sup> spores µl<sup>-1</sup>. In addition, solid media contain nutrients that may stimulate growth of microbial contaminants (Gu & Ko 1997). *Ampelomyces quisqualis* is of interest for commercial development because of its ability to control powdery mildew. Mixing *A. quisqualis* with paraffin, mineral oils or an additive (pinolene) made it more effective in controlling strawberry powdery mildew, a biological control strategy that can reduce conventional chemical fungicide residues (Ilaria et al. 2008). Furthermore, 2 bioactive metabolites from *Ampelomyces* spp. isolated from *Urospermum picroides* such as pyrone and sulfated anthraquinones were detected after growth in liquid or solid media. In addition, the metabolites 3-*O*-methylalaternin and altersolanol A showed effectiveness against the Gram-positive bacterium, *Staphylococcus aureus* which causes human disease (Amal et al. 2008).

Therefore, the objective of this study was screening of *Ampelomyces* spp. to assess their efficacy as biocontrol agents against powdery mildew of rose.

## Materials & Methods

### Isolates and morphology

#### Collection and isolation of *Ampelomyces* spp.

*Ampelomyces* spp. were isolated from four species of powdery mildew host plants: *Zinnia elegans* (Zinnia), *Ageratum conyzoides* (Chick weed), *Hydrangea* spp. (Hydrangea) and *Dahlia* spp. (Dahlia) which presented as brownish colonies on leaves. The samples were collected during November 2018- February 2019 from Chiang Mai and Chiang Rai province, Thailand (Table 1).

#### Morphology observation by light and scanning electron microscopy

*Ampelomyces* spp. were stripped off leaves by clear adhesive tap then pressed on glass slides following Cook et al. (1997) and To-anun & Takamatsu (2007) to investigate pycnidia shape and size by light microscopy using a 40X objective with a phase contrast lens. The colonies of *Ampelomyces* sp. on leaves were cut and fixed with FAA solution (ethanol 45%, v/v; acetic acid 6%, v/v; formaldehyde 5%, v/v and SDW 44%) following Pathan & Gaskin (2010) for observation of hyperparasitism by scanning electron microscopy (SEM) (JEOL 5410, Tokyo, Japan) (Fig. 1).

#### Effect of *Ampelomyces* sp. on spore germination of powdery mildew

All isolations of *Ampelomyces* spp. were done from leaf surfaces which were sterilized with 70% EtOH for 1 min and rinsed with sterile water. Pycnidia were then transferred for mass growth production in Wickerham liquid medium (3 g yeast extract, 3 g malt extract, 5 g peptone, 10g glucose, with addition of up to 1,000 ml of distilled water, pH adjusted to 7.2-7.4) following Amal et al. (2008), and incubated 25°C (Gu 1998), on a shaker 220 rpm for 45 d.

Four isolates of *Ampelomyces* spp., AMP1-Ze, AMP2-Ac, AMP3-Hg, AMP4-Dl were mass-produced in the liquid medium, on liquid medium. The biocompounds were extracted with ethanol

three times then evaporated and tested for conidial inhibition (LC50) on onion tissue using the methods of Hirata (1942) at concentrations of 0, 10, 50, 100, 500 and 1,000 ppm 48 hr. after treatment (4 treatments and 5 replications) (Table 2).

## Greenhouse experiments

### Effect of a biocompound on *P. pannosa* powdery mildew severity

All treatments were applied weekly for 4 wk and disease was estimated in the last week. A randomized complete block design (RCBD) with five replicates was used for data collection following Townsend & Heuberger (1943) and Biswas et al. (1992) Each treatment had 100 leaflets (20 leaflets/replicate) which were examined to assess the disease severity as the percentage of infected area based on a 1–5 scale divided by Townsend & Heuberger (1943) and Biswas et al. (1992) (Table 3, Fig. 2).

The powdery mildew disease severity was also assessed by scoring selected at random, on a scale of 1–5 (1 = 1–10%, 2 = 11–15%, 3 = 16–25%, 4 = 25–50% and 5 = >50% of leaf area infected with powdery mildew growth. The percent disease index (PDI) was calculated following Mckinney (1923) and the methods of Chiang et al. (2017) infection index.

$$\text{PDI} = \frac{\text{Sum of all individual ratings}}{\text{Total number of leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

The effectiveness of the treatments was calculated according to the following formula:

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

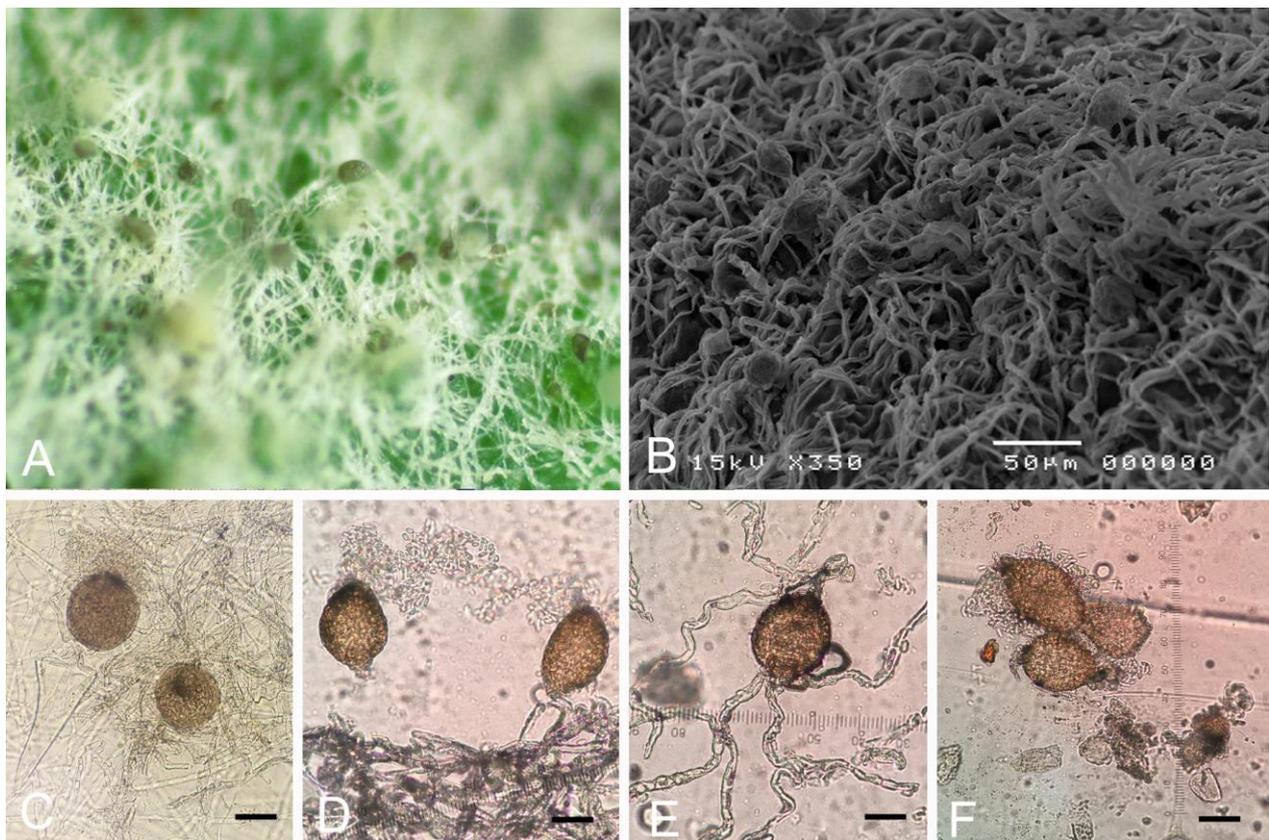
## Results

The hyperparasitic fungus *Ampelomyces* spp. was isolated from primary infections of powdery mildew that appeared on the leaf surfaces of four species of hosts: *Golovinomyces cichoraceanum*, *Podosphaera xanthii*, *Pseudoidium hortensiae* and *Golovinomyces cichoraceanum* (Table 1). Colonies presented a brownish colour. Morphological characteristics were observed by light microscope and included pycnidia which varied in shape (ovoid and ellipsoid) depending on the host fungus, and conidia measuring average approximately 10.5 to 13.5 µm in length x 2.5 to 3.0 µm in width. Scanning electron microscope (SEM) indicated that *Ampelomyces* spp. penetrated into powdery mildew mycelium (Fig. 1).

**Table 1** Screening *Ampelomyces* spp. from powdery mildew hosts.

Isolate Name	Host plants <sup>a</sup>	Powdery mildew host fungus	Area of isolation
AMP1-Ze	<i>Zinnia elegans</i>	<i>Podosphaera fusca</i> (Mukhtar & Arend 2017)	Mae-Sai, Chiang Rai
AMP2-Ac	<i>Ageratum conyzoides</i>	<i>Podosphaera xanthii</i> (Braun & Cook 2012)	Mae-on, Chiang Mai
AMP3-Hg	<i>Hydrangea</i> sp.	<i>Pseudoidium hortensiae</i> (Meeboon & Takamutsa 2015)	Bhubing Palace, Chiang Mai
AMP4-Dl	<i>Dahlia</i> spp.	<i>Golovinomyces cichoraceanum</i> (Braun & Cook 2012)	Bhubing Palace, Chiang Mai

<sup>a</sup> All specimens were deposited in the Mycological Herbarium in the Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Thailand.



**Fig. 1** – *Ampelomyces* spp. on powdery mildew. A Pycnidial hyperparasitism of powdery mildew on leaf surface. B Microscopic interactions between mycoparasites *Ampelomyces* and powdery mildew, SEM micrographs. C Pycnidia and conidia isolated from *Golovinomyces cichoraceanum*. D Pycnidia and conidia isolated from *Podosphaera xanthii*. E. Pycnidia and conidia isolated from *Pseudoidium hortensiae*. F Pycnidia and conidia isolated from *Golovinomyces cichoraceanum* Scale bars: A = 10 µm, B = 50 µm, C–F = 10 µm.

**Table 2** Effect of biocompounds of *Ampelomyces* spp. on inhibition of *Podosphaera pannosa* conidial germination.

Biocompounds	Growth Inhibition <sup>1,2</sup> (%) at each concentration (ppm)					
	10	50	100	500	1,000	LC <sub>50</sub> (ppm)
<i>Ampelomyces</i> sp. AMP1-Ze (EtOH)	20 <sup>d</sup>	29 <sup>d</sup>	40 <sup>c</sup>	60 <sup>b</sup>	75 <sup>a</sup>	190.55
<i>Ampelomyces</i> sp. AMP2-Ac (EtOH)	15 <sup>d</sup>	39 <sup>c</sup>	65 <sup>b</sup>	71 <sup>b</sup>	84 <sup>a</sup>	91.20
<i>Ampelomyces</i> sp. AMP3-Hg (EtOH)	12 <sup>e</sup>	41 <sup>d</sup>	56 <sup>c</sup>	68 <sup>b</sup>	83 <sup>a</sup>	109.64
<i>Ampelomyces</i> sp. AMP4-Dl (EtOH)	21 <sup>e</sup>	35 <sup>d</sup>	52 <sup>c</sup>	67 <sup>b</sup>	78 <sup>a</sup>	112.20

<sup>1</sup> Growth Inhibition (GI) = R1-R2/R1x100: R1 = Number of spores of the tested pathogen produced in the control (0 ppm) and R2 = Number spores of the tested pathogen produced at each concentration.

<sup>2</sup> The same letters following means in each column indicate that they are not significantly different using Duncan's multiple range test.

**Table 3** Effect of a biocompound on *P. pannosa* powdery mildew severity.

Treatments (application time) <sup>a</sup>	Powdery mildew severity reading level 1-5 <sup>b</sup>		
	First reading initial infection	Highest reading 4 wk	Efficiency% <sup>1</sup>
Control (non-treated)	1.52 <sup>a</sup>	4.72 <sup>a</sup>	-
<i>Ampelomyces</i> sp. AMP2-Ac (100 ppm)	1.66 <sup>a</sup>	2.58 <sup>b</sup>	45.33 <sup>b</sup>
Carbendazim (10cc/20L)	1.58 <sup>a</sup>	2.29 <sup>c</sup>	51.48 <sup>a</sup>

<sup>a</sup> Application times were weekly for 4 wk after initial powdery mildew infection.

<sup>b</sup> Mean disease severity reading (1 = 1–10%, 2 = 11–15%, 3 = 16–25%, 4 = 26–50% and 5 = >50% of leaf area infected with powdery growth); means followed by the same letters in column are not significantly different (LSD;  $P=0.05$ )

<sup>1</sup> The averages were calculated using data from 10 plants per each treatment (10 leaves/plant).



**Fig. 2** – Disease severity in greenhouse experiment after spray application at 4 wk. A Control (non-treated). B Biocompound extract from *Ampelomyces* sp. C Chemical fungicide (carbendazim).

## Discussion

The interaction of *Ampelomyces* spp. and powdery mildew in natural infections presents an opportunity most effective to control disease severity and develop a biological control product (Viterbo et al. 2007). The frequent application of a mycoparasite in the field or greenhouse was able to reduce powdery mildew severity (Diego et al. 2003). Successful infection of powdery mildew by *Ampelomyces* spp. occurs before or during rain events. This presents a difficulty for commercial plant management which can be overcome using spray equipment to create wet conditions (Stuart et al. 1995). Moreover, the relationship with its host fungi is also an important factor (Claudia et al. 2005). So, the use of biocompounds in field or commercial-scale control is less problematic and could be developed as formulations. In this study the highest efficiency most effective biocompound was extracted from the *Ampelomyces* isolate infecting *Ageratum conyzoides* (AMP2-Ac) which compared favourably to the conventional fungicide (carbendazim 10cc/20L) in reducing the severity of powdery mildew disease by 45.33% in greenhouse experiments. However, genetic diversity is needed to confirm the relationship between *Ampelomyces* mycoparasites and their hosts for control of powdery mildew on other host plants and to determine suitable timing for their use (Orsolya et al. 2005).

In conclusion, the results from this study indicated that biocompounds extracted from *Ampelomyces* spp. can inhibit powdery mildew conidial germination, and that the use of such biocontrol agents has potential for control of rose powdery mildew (Bélanger & Labbé 1994). It appears that growers need to spray frequently to reduce the risk of powdery mildew disease.

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