

## First Report of Powdery Mildew Caused By *Podosphaera xanthii* on *Ageratum conyzoides*, in Indonesia

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### Abstract.

Powdery mildew is one of the most common and important fungal diseases and may cause huge economic losses to crop yields worldwide. During survey of powdery mildews in 2011 and 2013, six samples of *Ageratum conyzoides* were collected from different regions in Indonesia, i.e. Bali, South Sumatera and West Java provinces. Anamorphic features revealed that the fungus belongs to the genus *Podosphaera*. Two sets of sequences of both ITS rRNA and 28S regions were obtained from the six samples. Phylogenetic analyses, including maximum parsimony (MP) and maximum likelihood (ML) were executed using MEGA7. The strength of internal branches of the resulting trees were tested with bootstrap analysis. Tree scores, including tree length, CI, RI and RC were also calculated. The phylogenetic analysis confirmed that the fungus belongs to the genus *Podosphaera*, forming a clade with the sequences from *Podosphaera xanthii*. This is the first report of powdery mildews on *Ageratum conyzoides* from Indonesia.

**Keywords :** Anamorph; Erysiphales; Molecular Phylogeny and Plant Disease.

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## I. INTRODUCTION

Powdery mildew is one of the most common and important fungal diseases on many economically important plants. It forms a white powdery film on the surface of plant leaves, stems, flowers, and fruits by producing quite large white mycelia and powdery spores like talcs. It is an obligate biotrophic ascomycetous fungi belonging to the order Helotiales comprising ca 900 species belonging to 18 genera (Shirouzu et al., 2020). They are able to colonize approximately 10.125 plant species belonging to 205 families (Glawe, 2008; Bradshaw et al., 2024).

Powdery mildews may cause extreme yield losses on some economically important plants and crops world-wide i.e. severe defoliation and reduction in size and number of fruits on chili (*Capsicum annum* L.) caused by *Leveillulla taurica* in India (Bademiya and Ashtaputre, 2019), premature defoliation and drying on mungbean (*Vigna radiata* L.) caused by *Erysiphe polygoni* in India (Meena et al., 2022), reduction in cone yield and quality on hop (*Humulus lupulus* L.) caused by *Podosphaera macularis* in USA (Gent et al., 2014), poor fruit set and low yield on grapes (*Vitis vinifera*) caused by *Uncinula necator* in Pakistan (Uddin et al., 2022).

There are very few reports regarding powdery mildews in Indonesia despite the rich diversity of plants. Most of those reports were mainly written based on the anamorphic features only, which sometimes were unreliable due to similarity among closely related species (Raciborski 1900, Palm 1921, Schwarz 1926, 1927, Schweizer 1928, Spaulding 1961, Reddy 1970, Hirata 1986 and Semangun 1992). Since 2011, extensive surveys of powdery mildews were carried out in several regions of Indonesia. The determination of powdery mildew species of Indonesia is now carried out by using combination of molecular study (nucleotide sequences

of the ribosomal DNA (rDNA) internal transcribed spacer (ITS) region and 28S region) as well as by light and scanning electron microscope (Meeboon et al., 2012a, 2012b, 2013a, 2013b); Siahaan et al., (2015, 2016a, 2016b, 2016c).

*Ageratum conyzoides* is a species native to Central and South America, belonging to the family Asteraceae. In Indonesia, this plant is considered a weed found along the roadsides and crop fields. During survey of powdery mildews in 2011, we collected two samples of *A. conyzoides* infected by powdery mildews from Bandung, West Java and one sample from Indralaya, South Sumatra. In 2013, we collected two more samples from Bandung, but from a different location from the first collection point and one sample from Bali. Identification of this fungus is crucial because this fungus could be a threat to economically important plants in the future.

## II. METHODS

### Fungal Collection and Morphological Observation

Samples used in this study were collected between 2011 and 2013 in Indonesia. These samples were deposited in Mie University Mycological Herbarium (MUMH, Tsu, Japan) and Herbarium Bogoriense (BO BRIN, Bogor, Indonesia) (BO Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN), Bogor, Indonesia (Table 1). Collection date, location, host plant species and accession numbers of the nucleotide sequences were given.

In order to examine the asexual morph on the herbarium specimens, mycelial colonies on a small piece of infected leaf were rehydrated by the method described by Shin and La (1993). All specimens were examined using a light microscope (Axio Imager; Carl Zeiss, Göttingen, Germany) with phase contrast using 10×, 20× and 40× objectives. Thirty conidiophores and conidia were measured for each examined specimen. The size and shape of conidia; nature of conidiophores, e.g. size and shape of foot-cells, position of basal septa, shape and position of hyphal appressoria, position of germ tubes of conidia; and shape of appressoria on germ tubes of conidia (if found) were documented. For the rehydrated samples, the width of conidia was multiplied by Blumer's factor according to Braun and Cook (2012).

### DNA extraction and PCR amplification

Whole-cell DNA was isolated from fresh or herbarium fungal specimens by the chelex method (Walsh et al., 1991; Hirata and Takamatsu, 1996). Mycelia were taken using a clean adhesive tape sized 5 x 5 mm, then put into 300 µl of 5% Chelex (Bio-Rad) in a 1.5 ml microcentrifuge tube. The chelex suspensions containing mycelia were incubated at 56°C for 15 minutes. After mixing vigorously, the extracts were incubated in boiling water for 8 min, mixed using vortex for about 10-15 seconds and then boiled again for another 8 min. Thereafter, the extracts were centrifuged at 15,000 rpm for 5 min. Later, the supernatant was transferred onto a new tube and kept in -20°C degrees until used for PCR amplification. The nucleotide sequences of the 5'-end of the 28S rRNA gene (including domains D1 and D2) and internal transcribed spacer (ITS) regions including the 5.8S rRNA gene were determined in this study. PCR reactions were conducted in a total reaction of 25 µl, including the following reagents:

H<sub>2</sub>O 5.25 µl, 2x KOD buffer 12.5 µl; 2 mM of each deoxyribonucleotide triphosphate (dNTPs) 5 µl; two primers (Table 3.2) (20 pmol/µl) @ 0.375 µl, KOD FX Neo polymerase (1.0 unit/µl) 0.5 µl (*Toyobo, Japan*) and DNA 1 µl. PCR reactions were conducted under the following thermal cycling conditions in a thermal cycler SP (TaKaRa, Kyoto, Japan): an initial denaturing step at 94°C for 2 min; thermocycling for 40 cycles, where each cycle consisted of 10 sec at 98°C followed by 30 sec at 60°C for annealing. A positive and negative controls of template DNA were included for each set of reactions. The PCR product was subjected to preparative electrophoresis in 1.5% agarose gel in TBE buffer. The amplicons were sent to Solgent Co. Ltd (Daejeon, South Korea) for sequencing. New sequences determined in this study were deposited in DNA Data Base of Japan DDBJ under the accession numbers LC898138 – LC898139.

### Phylogenetic analysis

Newly determined sequences were aligned with other sequences of the Erysiphaceae retrieved from DNA databases (DDBJ, EMBL, NCBI) using MUSCLE (Edgar 2004) implemented in MEGA7 (Kumar et al., 2016). Alignments were further manually refined using the MEGA7 program. Maximum parsimony (MP) and

maximum likelihood (ML) methods were used in the molecular phylogenetic analyses of particular powdery mildews in this study. MP analyses were performed in PAUP\* 4.b10 (Swofford 2002) with heuristic search option using the tree bisection-reconstruction (TBR) algorithm with 100 random sequence additions to find global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. Kishino-Hasegawa (KH) and Shimodaira-Hasegawa (SH) tests (Kishino and Hasegawa, 1989; Shimodaira and Hasegawa, 1999) were performed to determine whether a given dataset can significantly reject a constraint tree constructed based on a hypothesis. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analyses using 1K replications with step-wise addition option set as simple (Felsenstein, 1985). BS values higher than 70% are given. Tree scores, including tree length, consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated.

The ML analysis was executed using MEGA7 (Kumar et al., 2016). In this analysis, partial deletion was set as gap/missing data treatment with coverage cut off was set 95%. The ML analysis was performed using the best evolutionary method determined respectively to the dataset used, and the initial tree for ML was set automatically. The strength of internal branches of the resulting tree was tested with bootstrap analysis using 1K replications (Felsenstein, 1985).

### III. RESULT AND DISCUSSION

#### Result

**Mycelium** effused or patches, persistent to subevanescent; hyphae substraight to somewhat wavy, fairly uniform or sometimes irregular in width; **hyphal appressoria** indistinct, sometimes nipple shape, single; conidiophores erect, arising from the top of mother cell, single on a hyphal cell (78.3–) 84.4–168.2(–184) x (7.3–)8.8–12.2(–12.7)  $\mu\text{m}$  (average 121 x 10.5  $\mu\text{m}$ ), **foot-cells** cylindrical, (36.9–)41–65.7(–87.2) x (7.3–)8.8–12.2(–12.7)  $\mu\text{m}$  (average 52.7 x 10.5  $\mu\text{m}$ ), straight or occasionally slightly curved, sometimes constricted and swollen at bases, followed by 1–3 shorter cells, producing 2–7 conidia in chains with crenate edge, with a basal septum at the branching point of the mycelium; **conidia** ellipsoid-doliiform, sometimes cylindric, (21.8–)25.1–32.1 (–37.1) x (12.9–)14.3–18.3(–19.8)  $\mu\text{m}$  (average 28.7 x 16.6  $\mu\text{m}$ ), producing germination tube of *Fibroidium*-type, occasionally from terminal, alobatus.

#### Discussion

Tropical fungi have traditionally been under-researched and their taxonomic placement has been confounded, often misidentified with temperate fungi. Limkaisang et al., (2006) stated that the ecology and classification of the powdery mildew fungi on tropical trees are still uncertain, not only because of the limited number of researchers working on this fungal group in tropical regions but also the lack of teleomorphic state, which are necessary for species identification. Further, they stated that the identification of tropical powdery mildews fungi is mostly in their host plants and anamorphic state, which are not adequate to distinctly delimit the species. Moreover, Arnold (2011) emphasized that research on tropical fungi remains constrained by several factors: (i) the substantial training required to conduct sampling in complex tropical environments, (ii) the limited number of newly trained taxonomists specializing in tropical mycology, and (iii) the persistent challenges in defining clear species boundaries. In a similar vein, these constraints indicate that investigations of powdery mildews in Southeast Asian countries are also relatively limited. This situation is likely attributable to the comparatively small community of mycologists focusing on powdery mildews in the region, especially when contrasted with Europe, North America, and East Asia, as well as to the frequent absence of the sexual morph, which is essential for accurate species identification in subtropical and tropical regions.

There are very few reports regarding on powdery mildews in Indonesia. These reports were mainly written based on the conventional taxonomical system, based on anamorph and not supported by molecular data. However, over the past decade, the exploration of powdery mildews in Indonesia is rapidly increasing. By combining morphological, molecular and host range data, eight new species have been reported from this country since 2012 (Meeboon et al., 2012a, 2012b, 2013a, 2013b); Siahaan et al., (2015, 2016a, 2016b, 2016c). These reports indicated that Indonesia has potentially many unique, undescribed and probably endemic powdery mildew species, playing an important role in providing additional information of the tropical powdery mildews, especially from Indonesia, for a better understanding of the geographical distribution and evolution

of powdery mildews in the world. Therefore, the survey on the diversity of this group of fungi and its distribution in Indonesia should be carried out to provide a comprehensive database of the fungi based on the current generic and/or species concept.

PLANT NAME	Collection Date	LOCATION			BO BRIN No.	MUMH No.	ITS
		Place	LatNS	LonWE			
1. <i>Ageratum conyzoides</i>	2011.03.13	Patrol Belapati Cihideung, Parongpong, West Bandung	06°48'2 4.2"	107°35'1 9.4"	-	5140	O
2. <i>Ageratum conyzoides</i>	2011.03.13	Daerah Latihan Gunung Kopassus, Sekitar Situ Lembang, Bandung	06°40'5 2.9"	107°34'4 3.0"	-	5142	O
3. <i>Ageratum conyzoides</i>	2011.03.09	Indralaya, Palembang	03°17'4 7.3"	104°41'2 2.7"	-	5158	-
4. <i>Ageratum conyzoides</i>	2013.09.13	Desa Situ Patengang, Ciwidey, Bandung	07°09'5 5.7"	107°21'3 8.4"	22706	5680	-
5. <i>Ageratum conyzoides</i>	2013.09.14	Rawa Upas, Bandung	07°08'2 2.0"	107°23'2 5.3"	22710	5685	-
6. <i>Ageratum conyzoides</i>	2013.09.16	Gitgit Waterfall, Bali	08°12'1 3.4"	115°08'2 1.6"	22732	5707	-

**Table.1** –Fungal sample materials of *P. xanthii* collected in this study

The only information regarding powdery mildews on *Ageratum* in Indonesia was recorded by Aryuti and Rifai (1987) on *Ageratum houstonianum*, identified as *Oidium tabaci*. However, in their report, they did not provide any morphological or molecular information at all. Thus, the identification of this species is doubtful.

Three powdery mildews species were reported on *A. conyzoides* in the world, i.e. *Erysiphe cichoracearum*, *Oidium ageratii* and *Sphaerotheca fuliginea* (Amano, 1986; Braun, 1987). The morphology of the current fungus is mostly similar to the anamorphic state of *Sphaerotheca fusca* ( $\equiv$  *Podosphaera fusca*). Braun et al., (2011) divided *P. fusca* to *P. fusca* and *P. xanthii* based on morphological and molecular characteristics. Recently, occurrence of powdery mildews on *Ageratum* were recorded from China (Mukhtar and Arend 2017), India (Thite et al., 2017) identified as *P. xanthii* and from Thailand (Meeboon et al., 2018), identified as *Golovinomyces* sp.



**Fig 1.** Phylogenetic analysis of the ITS rRNA gene sequences for two sequences of *P.xanthii* on *A. conyzoides* and sequences from *P. xanthii*. This tree is one of the five equally parsimonious trees. Bootstrap (BS) value ( $\geq 70\%$ ) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches. Samples retrieved in this study were marked with \*(asterisks).



**Fig 2.** Anamorphic features of *Podosphaera xanthii* on *A. conyzoides*. A–C. Conidiophores. D. Conidia. E. Germ tube. Bars: 20 µm.

Two sequences of the powdery mildew on *A. conyzoides* in this study were aligned with the data matrix of *Podosphaera* from the previous report (Takamatsu et al., 2010). The phylogenetic tree (MP tree) shown in Fig. 1 indicates that genus *Podosphaera* on *A. conyzoides* has a sequence identical with *Oidium* sp. on *Verbena bonariensis* and *Podosphaera fusca* on *Lactuca indica*, *Physalis* sp., *Tussilago farfara*, *Boehmeria nivea* and *Verbena x hybrida*, all identified as *Podosphaera xanthii*, confirming the identity of the fungus correspond with the anamorphic data. This is the first report of powdery mildew on *A. conyzoides* in Indonesia.

#### IV. CONCLUSION AND SUGGESTIONS

*Podosphaera xanthii* has a wide host range of mainly herbaceous plants, including the family of Asteraceae, Balsaminaceae, Cucurbitaceae, Fabaceae, Solanaceae and Verbenaceae. Some reports indicate that there were huge economic losses to crop yield, quality and value of economically important crops caused by this fungus (Afshan et al., 2025; Kelly et al., 2021). Annual loss of crop yield caused by plant disease are estimated to be more than 30% worldwide (Savary et al., 2019). Adequate knowledge of crop losses caused by disease is important for appropriate strategic and tactical management decisions.

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