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Identification and fungicide sensitivity of the blue mold pathogen in postharvest-stored elephant garlic bulbs in Thailand

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Abstract

Blue mold disease is one of the most important postharvest diseases affecting garlic bulbs. In 2023, this disease was found on bulbs of elephant garlic [*Allium ampeloprasum* var. *ampeloprasum* (Borrer) Syme] in Chiang Mai Province, Thailand, during the postharvest storage period. Three fungal isolates were obtained and identified as *Penicillium allii* based on morphological characteristics and phylogenetic analysis of combined sequences of the internal transcribed spacer (ITS) of ribosomal DNA, *β*-tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II second largest subunit (*rpb2*) genes. In the pathogenicity test, garlic bulbs inoculated with the isolated fungi exhibited symptoms similar to those observed during the postharvest storage period. In the fungicide screening test, carbendazim, difenoconazole + azoxystrobin, and difenoconazole effectively completely inhibited this fungus at both half and recommended dosages, while the fungus showed insensitivity to captan and mancozeb. Additionally, double-recommended dosages of carbendazim, copper oxychloride, difenoconazole combined with azoxystrobin, and difenoconazole alone exhibited complete inhibition of the fungus. To the best of our knowledge, this is the first report of postharvest blue mold disease on elephant garlic bulbs caused by *P. allii* in Thailand. Furthermore, the results of the fungicide sensitivity screening could help in developing effective management strategies for controlling postharvest blue mold disease on elephant garlic bulbs caused by *P. allii*.

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Introduction

Garlic (*Allium* spp.), especially the bulb, is commonly consumed and valued for both culinary and medicinal purposes due to its nutritional richness and numerous beneficial bioactive compounds essential for human health[1–3]. Elephant garlic [*Allium ampeloprasum* var. *ampeloprasum* (Borrer) Syme], hardneck garlic [*A. sativum* var. *ophioscorodon* (Link) Döll], and softneck garlic (*A. sativum* var. *sativum* L.) are popular varieties that have been cultivated worldwide[4,5]. In 2022, global garlic production reached 2.91 million tons, valued at 3.43 billion USD. China was the largest producer, contributing 2.13 million tons (73% of world production), followed by India with 0.3 million tons, Bangladesh with 0.05 million tons, and Egypt with 0.03 million tons[6]. Myanmar is the top garlic producer in Southeast Asia followed by Thailand and Indonesia[6]. At every stage of growth, harvesting, and post-harvest storage, garlic is susceptible to various diseases caused by bacteria, fungi, and viruses[7–9]. Diseases can significantly damage garlic bulb production and quality[9,10]. Blue mold disease, caused by *Penicillium* species, is a common issue affecting garlic bulbs during both the cultivation process and postharvest storage[9,11–14]. This disease can lead to significant customer dissatisfaction and economic losses in garlic production worldwide[9,11,14,15].

In Thailand, the northern part is the main region for garlic cultivation[16]. Nowadays, elephant garlic is a significant vegetable crop extensively cultivated in Thailand. Thus, the area of plantations used for growing garlic has significantly increased in Thailand. However, the incidence and severity of diseases have also increased when plants are grown in suboptimal areas and unsuitable storage conditions. In 2023, blue mold disease caused by fungi was observed on elephant garlic bulbs during the storage period in Chiang Mai Province in Thailand, with a degree of incidence within the range of 20% to 30%. Importantly, there had been no prior reports of blue mold disease on elephant garlic bulbs in Thailand. Therefore, the objective of this study was to isolate the causal fungal agents of this disease. The isolated fungi were identified using both morphological and molecular data. Pathogenicity tests were conducted, and Koch's postulates were applied to assess the effects of the isolated fungi on asymptomatic elephant garlic bulbs. Moreover, the sensitivity of the isolated fungi to several commercial fungicides was investigated using solid culture techniques.

Materials and methods

Sample collection

Blue mold disease was observed on elephant garlic bulbs (*A. ampeloprasum* var. *ampeloprasum*) throughout the postharvest storage at 25 to 30 °C and 65% to 75% relative humidity

over a period of 7 to 14 d in Chiang Mai Province, northern Thailand in 2023 (March to April). Garlic bulbs exhibiting typical symptoms were collected from postharvest storage stores and shipped to the laboratory within 24 h. After being transferred to the laboratory, symptomatic bulbs were examined using a stereo microscope (Nikon H55OS, Tokyo, Japan) and stored in a plastic container with moist filter paper to promote fungal sporulation.

Fungal isolation and morphological study

Samples of bulb disease were processed to isolate the fungal causal agents. The single conidial isolation method described by Choi et al.[17] was used to isolate the causal fungi from the lesions. This process was conducted on 1.0% water agar containing 0.5 mg/L streptomycin. The individual germinated conidia were observed after incubation at 25 °C for 24–48 h and then transferred directly onto potato dextrose agar (PDA; CONDA, Madrid, Spain) supplemented with 0.5 mg/L streptomycin under a stereo microscope. Pure cultures were deposited in the Culture Collection of Sustainable Development of Biological Resources (SDBR) Laboratory, Faculty of Science, Chiang Mai University, Thailand. The characteristics of the fungal colonies, including colony morphology, pigmentation, and odor, were examined on PDA, Czapek yeast extract agar (CYA), and malt extract agar (MEA; Difco, France) after incubation in the dark for 7 d at 25 °C. Micromorphological characteristics were assessed using a light microscope (Nikon Eclipse Ni-U, Tokyo, Japan). The Tarosoft® Image Frame Work software was used to measure at least 50 samples for each anatomical structure (such as conidiophores, phialides, and conidia).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from the fungal cultures of each isolate that grew on PDA at 25 °C for 5 d, using a Fungal DNA Extraction Kit (FAVORGEN, Ping-Tung, Taiwan) according to the manufacturer's protocol. Amplification of the internal transcribed spacer (ITS) of ribosomal DNA, β-tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II second largest subunit (*rpb2*) genes using ITS5/ITS4[18], Bt2a/Bt2b[19], CF1/CF4[20], and RPB2-5F/RPB2-7CR[21], respectively. The PCR for these four genes was conducted in separate PCR reactions and consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s (ITS and *BenA*); 51 °C for 1 min (*CaM*) and 52 °C for 1 min (*rpb2*), extension at 72 °C for 1 min, and final extension at 72 °C for 10 min on a peqSTAR thermal cycler (PEQLAB Ltd., Fareham, UK). PCR products were checked on 1% agarose gel electrophoresis and purified using a PCR clean-up Gel Extraction NucleoSpin® Gel and a PCR Clean-up Kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. The purified PCR products were directly sequenced. Sequencing reactions were performed, and the above-mentioned PCR primers were employed to automatically determine the sequences in the Genetic Analyzer at the 1st Base Company (Kembangan, Malaysia).

Sequence alignment and phylogenetic analyses

The analysis of the ITS, *BenA*, *CaM*, and *rpb2* sequences was conducted with the use of similarity searches employing the BLAST program available at NCBI (http://blast.ddbj.nig.ac.jp/top-e.html, accessed on 10 July 2024). The sequences from this study and those obtained from previous studies, together with sequences downloaded from the nucleotide GenBank database are listed in Table 1. Multiple sequence alignment was performed with MUSCLE[22] and improved where necessary using BioEdit v. 6.0.7[23]. Finally, the combination datasets of ITS, *BenA*, *CaM*, and *rpb2* sequences were performed.

For phylogenetic analyses, *Penicillium italicum* (CBS 339.48) and *P. ulaiense* (CBS 210.92) were selected as the outgroup. The maximum likelihood (ML) analysis was carried out using RAxML-HPC2 version 8.2.12 on the GTRCAT model with 25 categories and 1000 bootstrap (BS) replications[24,25] *via* the CIPRES web portal. Bayesian inference (BI) analysis was performed with MrBayes v. 3.2.6 software for Windows[26]. The best substitution model for BI analysis was estimated using the jModelTest 2.1.10[27] by employing the Akaike information criterion (AIC). Bayesian posterior probability (PP) was determined by Markov Chain Monte Carlo Sampling (MCMC). Four simultaneous Markov chains were run for a million generations with random initial trees, wherein every 100 generations were sampled. The first 25% of generated trees representing the burn-in phase of the analysis were eliminated, while the remaining trees were used for calculating PP in the majority-rule consensus tree. The phylogenetic trees were visualized using FigTree v1.4.0[28].

Pathogenicity tests

Conidia from fungal isolates cultivated for two weeks on PDA were used in this experiment. Healthy commercial elephant garlic bulbs were washed thoroughly, and then their surfaces were sterilized by soaking them for 5 min in a sterile sodium hypochlorite solution with a concentration of 1.5% (*v*/*v*). Following that, sterile distilled water was used to wash them three times. The bulbs were allowed to air-dry at room temperature (25 ± 2 °C) for 10 min after surface disinfection. Following the air-drying process, a quantity of 10 µL of a conidial suspension (1×10^6 conidia/mL) in sterile water from each fungal isolate was dropped onto each bulb. Consequently, sterile distilled water was used as an inoculant for the control. The inoculated bulbs were placed in individual 4 L sterile plastic boxes maintained at 80% relative humidity. These containers were kept in a growth chamber at a temperature of 25 °C under a 12-h light cycle for one week. A total of ten replicates were used for each treatment, which was repeated twice under the same conditions. The disease symptoms were observed. Confirmation of Koch's postulates was achieved by re-isolating the fungi through the isolation method from any lesions that occurred on the inoculated bulbs.

Screening of commercial fungicides against the causal agent

Seven commercially available fungicides, including benalaxyl-M (4%) + mancozeb (65%) (Fantic M WG®, Thailand), captan (Captan 50®, Thailand), carbendazim (Dazine®, Thailand), copper oxychloride (Copina 85 WP®, Thailand), difenoconazole (12.5%) + azoxystrobin (20%) (Ortiva®, Thailand), difenoconazole (Score®, Thailand), and mancozeb (Newthane M-80®, Thailand), were examined in this study according to the approach indicated through previous studies[29,30]. The fungicides used in this study were available commercially in Thailand and were approved for usage. The *in vitro* applications of benalaxyl-M + mancozeb, captan, carbendazim, copper oxychloride, difenoconazole + azoxystrobin, difenoconazole, and mancozeb were recommended at dosages of 1,380, 750, 750, 1,700, 243.75, 187.5, and 1,200 ppm, respectively, according to the labels for each fungicide. The final concentration was

Table 1. Details of sequences in *Penicillium* section *Fasciculata* used in molecular phylogenetic analysis.

Penicillium species	Strain/isolate	GenBank accession number			
		ITS	*BenA*	*CaM*	*rpb2*
P. albocoremium	CBS 472.84^T	AJ004819	AY674326	KUJ896819	KU904344
P. allii	CBS 131.89^T	–	AY674331	KU896820	KU904345
P. allii	**SDBR-CMU499**	**PP998350**	**PQ032853**	**PQ032856**	**PQ032859**
P. allii	**SDBR-CMU500**	**PP998351**	**PQ032854**	**PQ032857**	**PQ032860**
P. allii	**SDBR-CMU501**	**PP998352**	**PQ032855**	**PQ032858**	**PQ032861**
P. aurantiogriseum	CBS 324.89	AF033476	AY674296	KU896822	JN406573
P. camemberti	MUCL 29790^T	AB479314	FJ930956	KU896825	JN121484
P. cavernicola	CBS 100540^T	MH862709	KJ834439	KU896827	KU904348
P. caseifulvum	CBS 101134^T	MH862722	AY674372	KU896826	KU904347
P. commune	CBS 311.48^T	AY213672	AY674366	KU896829	KU904350
P. concentricum	CBS 477.75^T	KC411763	AY674413	DQ911131	KT900575
P. coprobium	CBS 561.90^T	DQ339559	AY674425	KU896830	KT900576
P. discolor	CBS 474.84^T	OW986149	AY674348	KU896834	KU904351
P. echinulatum	CBS 317.48^T	MH856364	AY674341	DQ911133	KU904352
P. freii	CBS 476.84^T	MH861769	KU896813	KU896836	KU904353
P. gladioli	CBS 332.48^T	AF033480	AY674287	KU896837	JN406567
P. glandicola	CBS 498.75^T	AB479308	AY674415	KU896838	KU904354
P. griseofulvum	CBS 185.27^T	AF033468	AY674432	JX996966	JN121449
P. hirsutum	CBS 135.41^T	AY373918	AF003243	KU896840	JN406629
P. hordei	CBS 701.68^T	MN431391	AY674347	KU896841	KU904355
P. italicum	CBS 339.48^T	KJ834509	AY674398	DQ911135	–
P. melanoconidium	CV1331	JX091410	JX091545	JX141587	KU904358
P. neoechinulatum	CBS 101135^T	JN942722	AF003237	KU896844	JN985406
P. nordicum	DTO 098-F7	KJ834513	KJ834476	KU896845	KU904359
P. palitans	CBS 107.11^T	KJ834514	KJ834480	KU896847	KU904360
P. polonicum	CBS 222.28^T	AF033475	AY674305	KU896848	JN406609
P. solitum	CBS 424.89^T	AY373932	AY674354	KU896851	KU904363
P. thymicola	CBS 111225^T	KJ834518	AY674321	FJ530990	KU904364
P. tricolor	CBS 635.93^T	MH862450	AY674313	KU896852	JN985422
P. ulaiense	CBS 210.92^T	KC411695	AY674408	KUB96854	KU904365
P. verrucosum	CBS 603.74^T	AY373938	AY674323	DQ911138	JN121539
P. vulpinum	CBS 126.23^T	AF506012	KJ834501	KU896857	KU904367

Ex-type species are indicated by the superscript letters as 'T'. '–' indicates the absence of sequencing information in GenBank. The fungal isolates and sequences obtained in this study are in bold.

obtained by preparing each fungicide and adding it to an autoclaved PDA. Each fungicide was used in three different dosages: half-recommended, recommended, and double-recommended. The test media were inoculated with mycelial plugs (5 mm in diameter) that had been cultivated on PDA in the dark at 25 °C for one week. The control did not add any fungicide. The plates were maintained in darkness at a temperature of 25 °C. Following one week of incubation, the mycelial growth of each isolate was evaluated on individual plates and a comparison was made between the growth in PDA supplemented with fungicides and the growth observed in the control. The calculation of the percentage growth inhibition for each isolate was performed using the formula provided by Pandey et al.[31]. Each isolate was classified as sensitive (> 50% inhibition), insensitive (< 50% inhibition), or totally inhibited (100% inhibition) based on their growth inhibition rates[30,31]. Five replicates were conducted for each fungicide and fungal isolate, and the experiments were independently repeated twice under the same biological conditions.

Statistical analysis

The Shapiro-Wilk test in SPSS software version 26 was used to examine data from the two repeated fungicide sensitivity experiments at a significant level of $p < 0.05$ to perform the normality test. The results indicated non-significant findings, so the data from these repeated experiments were assessed for the assumptions of one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was then used to identify significant differences at $p \leq 0.05$.

Results

Disease symptoms

Initial symptoms, water-soaked areas on the outer surface of scales were observed. Later, white mycelium and blue powdery mold develop on the surface of the lesions (Fig. 1a). These lesions appear as brown, tan, or grey colored areas when the bulbs are cut. In advanced stages, infected bulbs disintegrated into a watery rot.

Fungal isolation and morphological characteristics

Three fungal isolates (CMU499, CMU500, and CMU501) with similar morphology were obtained and deposited at the SDBR-CMU under accession numbers SDBR-CMU499, SDBR-CMU500, and SDBR-CMU501, respectively. Colonies PDA, CYA, and MEA were 29–32, 32–37, and 33–37 mm in diameter, respectively after incubation for one week at 25 °C (Fig. 1b–d). Colonies on

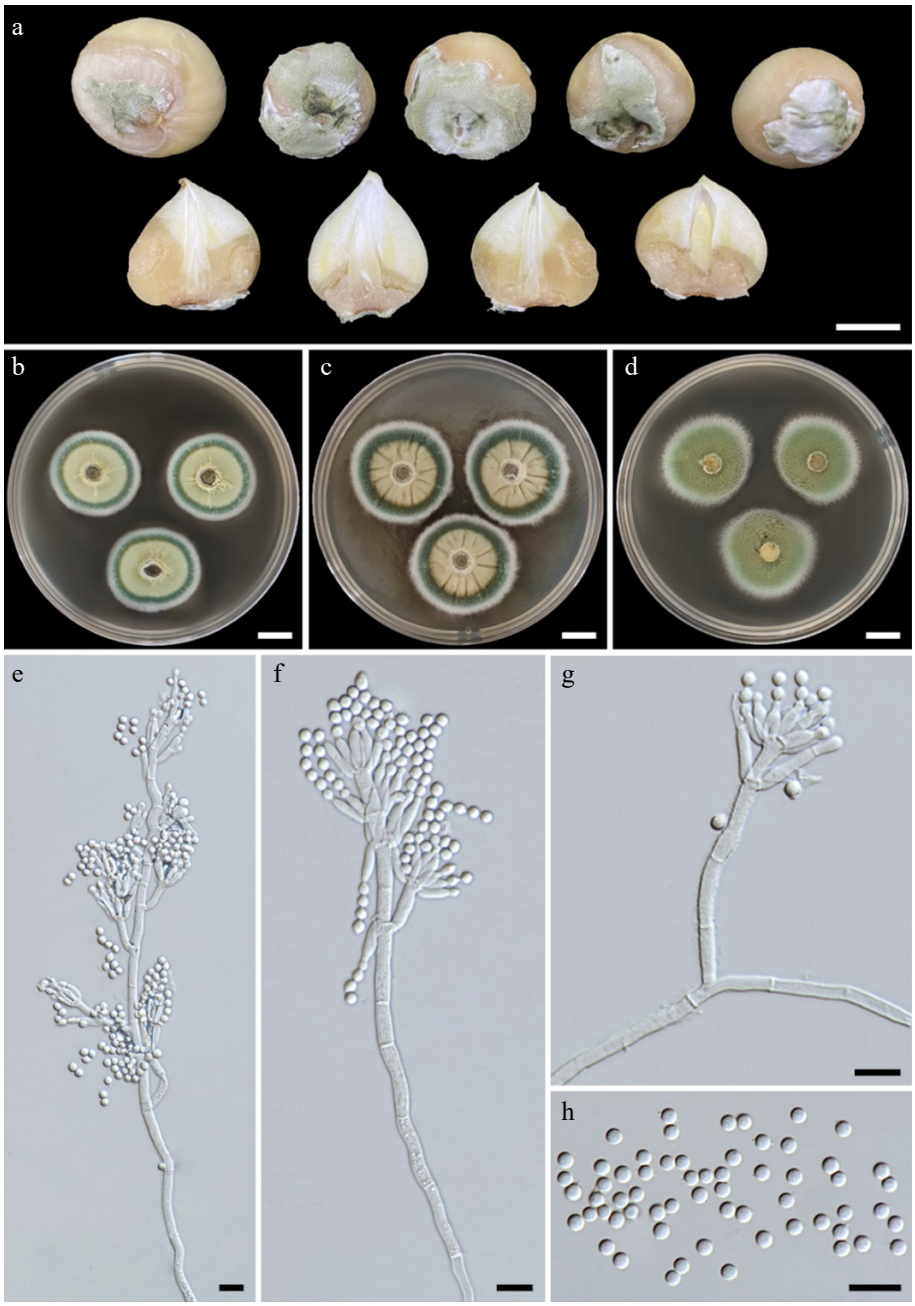

Fig. 1 (a) Natural symptoms of blue mold disease on bulbs of elephant garlic by *Penicillium allii* . Colonies of *Penicillium allii* SDBR-CMU499 after incubation at 25 °C for one week. (b) PDA. (c) CYA. (d) MEA. (e)–(g) Conidiophores. (h) Conidia. Scale bars: (a)–(d) = 10 mm, (e)–(h) = 10 µm.

PDA and MEA were white, flat with entire edges, conidium turquoise, white in the center, dull green at the margins; reverse pale yellow for PDA and yellow-brown for MEA. Colonies on CYA were white, flat with entire edges, conidium dull green; reverse white. All fungal isolates could produce conidiophores, and phialides, and sporulate in all of the agar media. *Conidiophores* terverticillate (Fig. 1e–g). *Stipes* rough-walled, 13.2–181.2 × 2.3–3.9 µm. *Rami* one or two, rough-walled and appressed or divergent, 8.4–24.7 × 2.5–4.6 µm. *Metulae* divergent, in verticils of 2–4, 8–19.1 × 2.3–4.6 µm. *Phialides* ampulliform, in verticils of 3 to 9, 6–17.9 × 1.7–6.9 µm. *Conidia* globose, 2.6–4.3 µm in diameter, smooth-walled, dull green (Fig. 1h). Based on these morphological characteristics, all fungal isolates were initially identified as belonging to *Penicillium*[32–35]. Fungal identification was then further confirmed using multi-gene phylogenetic analyses.

Phylogenetic analysis

The ITS, *BenA*, *CaM*, and *rpb2* sequences obtained from three fungal isolates in this study have been deposited in GenBank (Table 1). According to the BLAST results, all fungal isolates were identified as members of the *Penicillium* section *Fasciculata*. The combined ITS, *BenA*, *CaM*, and *rpb2* sequences dataset consists of 32 taxa, and the aligned dataset includes 2,399 characters comprising gaps (ITS: 1–553, *BenA*: 554–927, *CaM*: 928–1,442, and *rpb2*: 1,443–2,399). The best-scoring RAxML tree was established with a final ML optimization likelihood value of

–9,279.455311. Accordingly, the matrix contained 612 distinct alignment patterns with 5.04% undetermined characters or gaps. The estimated base frequencies were found to be: A = 0.235164, C = 0.269054, G = 0.262373, and T = 0.233408; substitution rates AC = 1.298254, AG = 4.466196, AT = 1.340638, CG = 0.787838, CT = 9.362594, and GT = 1.00000. The values of the gamma distribution shape parameter alpha and the Tree-Length were 0.580385 and 0.524602, respectively. Additionally, BI analysis yielded a final average standard deviation of 0.002257 for the split frequencies at the end of all MCMC generations. Regarding topology, the phylogenetic trees generated from both ML and BI analyses were similar. Consequently, the phylogenetic tree obtained from the ML analysis was selected and is displayed in Fig. 2. The results indicated that three fungal isolates SDBR-CMU499, SDBR-CMU500, and SDBR-CMU501 clustered with *P. allii* CBS 131.89 (ex-type strain) with strong statistical (100% BS and 1.0 PP) supports. Therefore, all fungal isolates obtained in this were identified as *P. allii* based on morphological and molecular data.

Pathogenicity tests

The initial symptoms appeared on bulbs of elephant garlic 3 d after being inoculated. After 7 d, all inoculated bulbs displayed powdery mold at their centers, surrounded by orange-brown water-soaked lesions (Fig. 3b–d). Whereas, control bulbs were asymptomatic (Fig. 3a). *Penicillium allii* was consistently reisolated from the inoculated bulbs on PDA to complete Koch's postulates.

Reactions of commercial fungicides against *Penicillium allii*

Seven commercially available fungicides in Thailand were tested in this study. After one week, the mycelial growths in response to the fungicides at three different dosages, including half-recommended (1/2RD), recommended (RD), and double-recommended (2RD) were calculated and presented in Table 2. The results revealed that the growth inhibition values varied across different fungicides, dosages, and fungal isolates. Data on the percentage of mycelial inhibition for each fungal isolate, related to the fungicides, passed the normality test (Shapiro-Wilk test, $p < 0.001$), thereby assuming normal distributions. Therefore, ANOVA followed by DMRT ($p \leq 0.05$) was used to identify significant differences. The findings indicated that all fungal isolates were completely inhibited by carbendazim, difenoconazole + azoxystrobin, and difenoconazole at all tested dosages (Table 2). In the tests for captan and mancozeb, all isolates demonstrated sensitivity to 2RD. Therefore, based on the recommended dosages, carbendazim, difenoconazole + azoxystrobin, and difenoconazole could be effectively applied to control this pathogen.

Discussion

Penicillium species are widely recognized as one of the most significant genera, known to cause major diseases in numerous economically valuable crops cultivated worldwide, including garlic[9,10,12,13,36]. Traditionally, *Penicillium* species have been identified using both macromorphological and micromorphological characteristics. However, morphological traits alone are insufficient to differentiate closely related *Penicillium* species due to the extensive range of morphological variations. Therefore, molecular techniques are crucial for accurately identifying *Penicillium* at the species level. Several previous studies have utilized a combination of ribosomal DNA (ITS) and protein-coding genes (*BenA*, *CaM*, *rpb1*, and *rpb2*) as powerful tools to identify *Penicillium* species since species-level identification remained unresolved when used solely on the ribosomal DNA gene[32,34,35,37]. In this study, three isolates of *P. allii* were obtained from the rot lesions of blue mold disease on elephant

Table 2. Percentage of mycelial inhibition and reactions of three isolates of *Penicillium allii* against fungicides.

Fungicides	Dosages	Inhibition of mycelial growth (%)*			Reaction
		SDBR-CMU499	SDBR-CMU500	SDBR-CMU501	
Benalaxyl-M + mancozeb	1/2RD	30.08 ± 1.41 c	29.27 ± 2.25 c	30.08 ± 2.53 c	Insensitive
	RD	55.28 ± 1.41 b	56.10 ± 3.62 b	60.16 ± 1.67 b	Sensitive
	2RD	82.11 ± 2.82 a	83.74 ± 1.41 a	83.74 ± 3.45 a	Sensitive
Captan	1/2RD	2.44 ± 3.50 c	1.63 ± 2.41 c	2.44 ± 1.25 c	Insensitive
	RD	4.88 ± 2.25 b	4.88 ± 2.23 b	5.69 ± 2.30 b	Insensitive
	2RD	72.36 ± 1.60 a	73.93 ± 3.45 a	73.98 ± 2.82 a	Sensitive
Carbendazim	1/2RD	100 ± 0 a	100 ± 0 a	100 ± 0 a	Inhibition
	RD	100 ± 0 a	100 ± 0 a	100 ± 0 a	Inhibition
	2RD	100 ± 0 a	100 ± 0 a	100 ± 0 a	Inhibition
Copper oxychloride	1/2RD	61.79 ± 1.60 c	58.54 ± 1.05 c	56.91 ± 1.41 f	Sensitive
	RD	68.29 ± 1.20 b	68.29 ± 2.05 b	68.29 ± 2.54 d	Sensitive
	2RD	100 ± 0 a	100 ± 0 a	100 ± 0 a	Inhibition
Difenoconazole + azoxystrobin	1/2RD	100 ± 0 a	100 ± 0 a	100 ± 0 a	Inhibition
	RD	100 ± 0 a	100 ± 0 a	100 ± 0 a	Inhibition
	2RD	100 ± 0 a	100 ± 0 a	100 ± 0 a	Inhibition
Difenoconazole	1/2RD	100 ± 0 a	100 ± 0 a	100 ± 0 a	Inhibition
	RD	100 ± 0 a	100 ± 0 a	100 ± 0 a	Inhibition
	2RD	100 ± 0 a	100 ± 0 a	100 ± 0 a	Inhibition
Mancozeb	1/2RD	19.51 ± 2.44 c	22.76 ± 3.73 c	21.14 ± 1.41 c	Insensitive
	RD	47.15 ± 1.45 b	47.15 ± 2.82 b	43.90 ± 2.44 b	Insensitive
	2RD	58.54 ± 2.44 a	54.47 ± 1.42 a	55.28 ± 1.45 a	Sensitive

* Results are means of five replicates ± standard deviation with the independently repeated twice. Data with different letters within the same column for each fungal isolate and fungicide indicate a significant difference at $p \leq 0.05$ according to Duncan's multiple range test. 1/2RD, RD, and 2RD indicate half of the recommended dosage, recommended dosage, and double the recommended dosage, respectively.

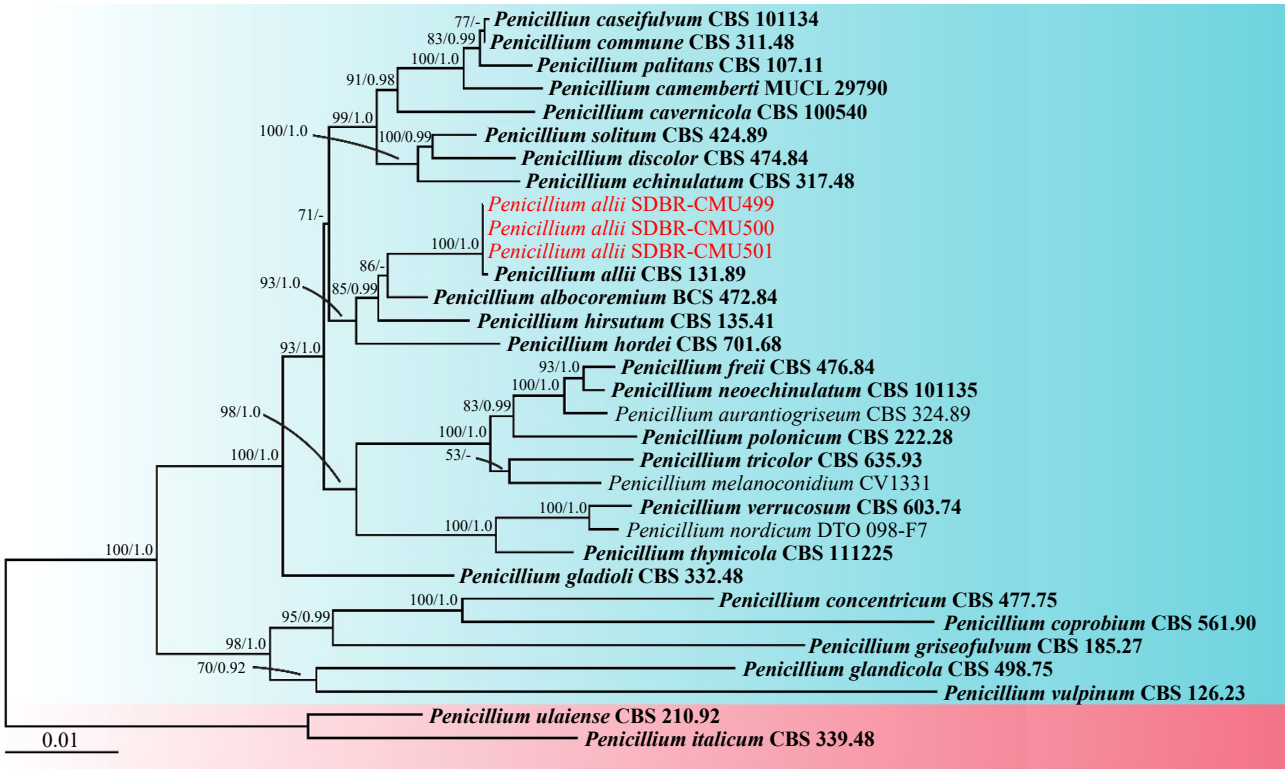

Fig. 2 Phylogram derived from maximum likelihood analysis of the combined ITS, *BenA*, *CaM*, and *rpb2* sequences of 30 taxa in the *Penicillium* section *Fasciculata* and two taxa in the *Penicillium* section *Penicillium*. *Penicillium italicum* CBS 339.48 and *P. ulaiense* CBS 210.92 were used as outgroups. Bootstrap values ≥ 50% (left) and Bayesian posterior probabilities ≥ 0.90 (right) are displayed above nodes. The scale bar represents the expected number of nucleotide substitutions per site. The sequences of fungal species obtained in this study are in red. The ex-type strain are in bold.

Fig. 3 Pathogenicity test using *Penicillium allii* SDBR-CMU499, SDBR-CMU500, and SDBR-CMU501 on bulbs of elephant garlic after one week inoculation at 25 °C. (a) Control bulbs treated with sterile distilled water instead of inoculum. Blue mold disease on bulbs of elephant garlic after inoculation of isolate (b) SDBR-CMU499, (c) SDBR-CMU500, and (d) SDBR-CMU501. Scale bar: 10 mm.

garlic bulbs in northern Thailand. The identification of this fungal species followed methods similar to those used for identifying *Penicillium*, which involve combining phylogenetic analysis of multiple genes with their morphological characteristics.

In this study, Koch's postulates were fulfilled by conducting pathogenicity tests on all isolates of *P. allii*. The findings demonstrate that postharvest blue mold disease on elephant garlic bulbs in northern Thailand, caused by *P. allii* identified in

this study resembles the disease caused by previously identified *Penicillium* pathogens, particularly *P. hirsutum*, which affects garlic bulbs worldwide[11–13,36]. *Penicillium polonicum* has been reported as a causal agent of blue mold on stored garlic bulbs in Pakistan[38]. *Penicillium allii* was known to cause postharvest blue mold disease on garlic bulbs in Argentina[15,39,40]. In the USA, *P. albocoremium*, *P. expansum*, *P. glabrum*, *P. paraherquei*, and *P. radicicola* can cause blue mold on garlic bulbs[13]. In Korea, blue mold disease on garlic bulbs caused by *P. hirsutum* has been reported[41]. Five *Penicillium* species, namely *P. allii*, *P. glabrum*, *P. italicum*, *P. polonicum*, and *P. psychrotrophicum* were identified and confirmed as postharvest pathogens causing blue mold rot of garlic in Serbia[42]. Recently, *P. allii* was the most virulent pathogen causing blue mold disease of elephant garlic bulbs in Italy, accounting for 95% of cases, followed by *P. citrinum* (4%) and *P. brevicompactum* (1%)[43]. Before this study, there were no reports of blue mold disease on elephant garlic bulbs in Thailand. Thus, this represents the first report of postharvest blue mold disease on elephant garlic bulbs caused by *P. allii* in Thailand.

To manage and control fungal-caused plant diseases, a variety of fungicides have been used. Several studies have documented the effectiveness of fungicides in affecting sensitive, resistant strains of plant pathogenic fungi, particularly those in the *Penicillium* species, on their *in vitro* mycelial growth[44–46]. In this study, the sensitivity and inhibition of *P. allii* to fungicides varied among different fungicides and dosages. These findings are consistent with previous studies, which reported that the sensitivity and inhibition of *Penicillium* species to fungicides varies based on the type and dosage of the fungicide, as well as fungal species[46–48]. Before this study, prochloraz had been used against *P. allii* to control diseases related to sprouting germination in Europe[49]. In this study, carbendazim, difenoconazole + azoxystrobin, and difenoconazole at both half and recommended dosages exhibited complete inhibition of *P. allii*. The information on the *in vitro* inhibition, sensitivity, and resistance of fungicides against *P. allii*, which causes postharvest blue mold disease on elephant garlic bulbs, would be beneficial for *in vivo* applications and for managing this disease in Thailand and globally. However, environmental factors and the fungicide's metabolism in the plant can cause the results of *in vitro* fungicide testing to differ from *in vivo* responses. Therefore, further studies are required to conduct *in vivo* fungicide sensitivity and disease inhibition assays based on the *in vitro* findings. Additionally, several previous studies have established that fungicide-resistant strains are a result of both excessive and prolonged fungicide treatment[50–52]. Utilizing biological control agents, rotating crops, adhering to fungicide treatment guidelines, and maintaining cleanliness in fields, equipment, and storage spaces are all essential components of a comprehensive strategy to reduce fungicide resistance in fungi[9,50,53,54].

Conclusions

Garlic blue mold disease, caused by *Penicillium* species, leads to significant economic losses during postharvest storage worldwide. In the present study, *P. allii* was isolated from infected bulbs of elephant garlic in northern Thailand. The identification of this fungi involved the analysis of their morphological characteristics and conducting multi-gene phylogenetic analyses. The assessment of pathogenicity for *P. allii* showed similar symptoms throughout the artificial inoculation process, as observed during the postharvest storage period. Therefore, this study represents the first report of elephant garlic blue mold disease caused by *P. allii* in Thailand. In the fungicide screening test, carbendazim, difenoconazole + azoxystrobin, and difenoconazole were found to effectively control this pathogen at both half and full recommended dosages. Thus, half of the recommended dosages can be used in managing this disease, serving as a guideline for prevention and helping to reduce pathogen resistance to fungicides. The findings of this study will enhance our understanding of postharvest blue mold disease in elephant garlic bulbs and provide insights for developing effective management strategies and prevention methods to minimize significant economic losses. Further research on the epidemiology of this disease would be required for effective monitoring, prevention, and control.

Author contributions

The authors confirm contribution to the paper as follows: conceptualization: Suwannarach N, Khuna S; formal analysis: Suwannarach N, Khuna S, Chaiwong K, Senwanna C, Kumla J; investigation, methodology: Suwannarach N, Khuna S, Chaiwong K; resources: Suwannarach N, Khuna S, Chaiwong K; software: Khuna S, Chaiwong K, Senwanna C, Kumla J; validation: Suwannarach N, Khuna S, Senwanna C, Nuangmek W; data curation: Khuna S, Senwanna C, Nuangmek W, Kumla J; visualization: Khuna S, Chaiwong K; writing–original draft: Suwannarach N, Khuna S, Nuangmek W, Kumla J; writing–review & editing: Suwannarach N, Khuna S, Chaiwong K, Senwanna C, Nuangmek W, Kumla J; supervision, project administration: Suwannarach N; funding acquisition: Suwannarach N, Nuangmek W. All authors have read and agreed to the published version of the manuscript.

Data availability

The DNA sequences generated in this study have been submitted to GenBank and can be accessed through the accession numbers provided in the paper.

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Conflict of interest

The authors declare that they have no conflict of interest. Nakarin Suwannarach and Jaturong Kumla are the Editorial Board members of *Studies in Fungi* who are blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer review handled independently of these Editorial Board members and the research groups.

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