International Journal of Agricultural Technology 2025 Vol. 21(4):1577-1596
DOI: https://doi.org/10.63369/ijat.2025.21.4.1577-1596
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Fungicide resistance of chrysanthemum fungal pathogens and control of leaf spot disease in pot conditions using effective fungicides

Tongsri, V.[1*], Apithanasakulngeon, P.[1], Songkumarn, P.[1], Suttiviriya, P.[2] and Chanchula, N.[3]

[1]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand; [2]Division of Agricultural Technology, Faculty of Science and Arts, Burapha University, Chanthaburi, Thailand; [3]Thailand Institute of Scientific and Technological Research, Pathum Thani, Thailand.

Tongsri, V., Apithanasakulngeon, P., Songkumarn, P., Suttiviriya, P. and Chanchula, N. (2025). Fungicide resistance of chrysanthemum fungal pathogens and control of leaf spot disease in pot conditions using effective fungicides. International Journal of Agricultural Technology 21(4):1577-1596.

Abstract Leaf spot and wilt are the most destructive diseases affecting chrysanthemum crops in Thailand. The results revealed that two leaf spot pathogens were similar to *Stemphylium lycopersici* and *Epicoccum sorghinum*, while wilt pathogens were close to *Fusarium solani* and *F. oxysporum*. Among all the tested fungicides, *S. lycopersici* was sensitive to iprodione, whereas *E. sorghinum* was found to be susceptible to three fungicides: iprodione, difenoconazole, and mancozeb. Of the soilborne fungi, *F. solani* was sensitive only to chlorothalonil, whereas *F. oxysporum* was sensitive to four fungicides: chlorothalonil, difenoconazole, copper oxychloride, and mancozeb. The two fungicides that effectively controlled leaf spots caused by *E. sorghinum* on detached leaves were difenoconazole (1,000 ppm) and mancozeb (2,000 ppm), with 76.9 and 84.6% disease control, respectively. Furthermore, mancozeb (2,000 ppm) greatly suppressed the disease in pot conditions by 93.9%. This finding indicated that mancozeb is an effective fungicide for further use in rotation with difenoconazole as a part of the chrysanthemum disease management program in Nakhon Ratchasima and Loei provinces.

Keywords: Chrysanthemum disease control, Chrysanthemum pathogen, Fungicide sensitivity

Introduction

Chrysanthemums are essential ornamental flowers that can be grown in many countries and in all regions of Thailand. Their prolific flowering with many varieties and colors has driven the strong customer demand for cut flowers and flowering pot plants. Major losses in the quantity and quality of chrysanthemum production worldwide have been due to fungal diseases. For example, leaf spot and blight diseases caused by two fungi, *Alternaria alternata* and *Nigrospora*

[*]**Corresponding Author:** Tongsri, V.; **Email:** fagrvnt@ku.ac.th

sphaerica, have been widely distributed in China (Luo *et al*., 2022a, b). Anthracnose disease has been reported in India, occurring on leaves and caused by *Colletotrichum siamense* (Gupta *et al*., 2023). Gray mold disease has been recorded, caused by *Botrytis cinerea* and appearing on diverse chrysanthemum plant parts (Sánchez-Pale *et al*., 2022). Chrysanthemums are sensitive to the rust fungus, *Puccinia horiana*, a major pathogen of this plant species in many growing areas (Chen *et al*., 2023; Munilakshmi *et al*., 2023). Chrysanthemum crops have also been targeted by *Didymella glomerata*, a leaf blight pathogen of maize (Afandi *et al*., 2022). It has been reported that soilborne diseases caused by diverse genera of pathogens, such as *Phytophthora*, *Phytopythium*, and *Fusarium*, could be causing disease in chrysanthemums (Afandi *et al*., 2022; Beaulieu *et al*., 2022; Miao *et al*., 2023). In addition, chrysanthemum plants are frequently infected by plant parasitic nematodes (Ding *et al*., 2019; Sert Çelik *et al*., 2019; Wang and Chen, 2020; Ali, 2023) as well as viruses, viroids and phytoplasmas (Contaldo *et al*., 2021; Bang *et al*., 2022; Supakitthanakorn *et al*., 2022; Walia *et al*., 2022; Navarro *et al*., 2023).

Various management strategies have been recommended for chrysanthemum disease control. For example, biological control is an important control agent for several diseases such as bacterial leaf blight, rust, and Fusarium wilt (Chen *et al*., 2018; Yusuf *et al*., 2019; Nuryani *et al*., 2021, 2022). Applying organic matter combined with the fumigant dazomet has reportedly enhanced the control of Fusarium wilt (Zhao *et al*., 2016). A foliar spray of micronutrient copper oxide has also minimized Fusarium wilt development (Elmer *et al*., 2021). Chrysanthemum-resistant varieties and transgenic plants have been proposed in rust management (Bety and Pangestuti, 2021; Sjahril *et al*., 2022). Additionally, induced resistance against foliar diseases has been demonstrated by various natural products and chemical inducers (Stapel and Guerrand, 2010; Kumar *et al*., 2020; He *et al*., 2023). However, effective fungicides have been often applied for decades to manage chrysanthemum diseases. For example, multisite fungicides, such as maneb, mancozeb, chlorothalonil, and captafol, minimized the severity of petal blight caused by fungal pathogens (Smith, 1966; Singh and Milne, 1974a). Single-site fungicides, such as demethylation inhibitor (DMI) and quinone outside inhibitor (QoI) fungicides or their mixtures, provided notable control of rust disease (Dickens, 1990; Lam and Lim, 1993; Wojdyła, 2006, 2007). Three other fungicides (iprodione, hexaconazole, and carbendazim) mixed with mancozeb, provided major control of Septoria leaf spots (Chandel and Chandel, 2010). Captan and thiophanate-methyl significantly lessened chrysanthemum cutting rots (Satou *et al*., 2013).

The prolonged use of fungicides possibly causes adverse effects on controlling plant diseases because it could lead to the emergence of fungicide-

resistant pathogens. In chrysanthemums, *P. horiana* is strongly resistant to the QoI fungicide azoxystrobin (Matsuura, 2019; Matsuzaki *et al*., 2021a,b). The sensitivity of basidiospore germination of *P. horiana* to fungicides has been found at high EC_{50} values of >9,000 parts per billion (ppb) for DMIs and >200 ppb for multisite fungicides (Palmer *et al*., 2015).

The *in vitro* mycelial growth of *F. avenaceum,* the wilt pathogen of chrysanthemums, was sensitive to DMI and methyl benzimidazole carbamate (MBC) fungicides but insensitive to multisite fungicides (Kopacki and Wagner, 2006). Singh and Miline (1974b) found that the fungal complex of the petal blight pathogens, *Alternaria*, *Botrytis*, *Itersonilia*, and *Stemphylium*, were sensitive to multisite fungicides with low EC_{50} values (1–5 ppm). Most single-site fungicides are prone to provide possible resistance to the pathogens based on high EC_{50} values (greater than or equal to 100 ppm). Fortuitously, Ishii *et al*. (2022) reported that the anthracnose pathogen *C. chrysanthemi* was still sensitive to enzovindiflupyr, a succinate dehydrogenase inhibitor (SDHI) fungicide. Hence, it could be applied to chrysanthemum pots to control the disease.

In Thailand, there is no up-to-date identification of chrysanthemum fungal pathogens. In addition, fungicide-resistant tests of the pathogens have been rarely reported. Therefore, the objectives of this research were to investigate the molecular identification of chrysanthemum pathogens, to demonstrate the resistance of pathogens to ten fungicides (six single-site fungicides and four multisite fungicides), and to evaluate the effective fungicides for controlling diseases based on detached leaf and potted plant experiments.

Materials and methods

Diseased sample collection

Chrysanthemum plantation areas in southeastern Thailand in three provinces (Loei, Nakhon Ratchasima, and Ubon Ratchathani) were selected for this study. Two chrysanthemum fields in each province were randomly selected. Plants exhibiting leaf spot and wilt symptoms were collected in each field, kept in ice boxes, and delivered to the laboratory within 48 h. The whole plant was collected where wilt symptoms were evident.

Pathogen isolation

The tissue transplanting technique was used to isolate pathogens by cutting the leaf spot areas into 0.5 cm^2 pieces, or root and stem rots into 0.5 cm lengths. The diseased tissues were surface-sterilized with 1% sodium hypochlorite for 7

min, rinsed twice with sterile distilled water, and dried in a laminar flow cabinet. Then, sterilized diseased tissues were transferred to potato dextrose agar (PDA) plates and incubated at room temperature (25±2°C) for 5–7 days. The fungal mycelia grown from diseased tissues were cut at the colony border. The pure culture was maintained using the hyphal tip technique and kept on a PDA slant for further use.

Pathogenicity test

A 0.5 cm diameter agar plug of a folia pathogen culture aged 7 days of each fungal isolate was inoculated on detached chrysanthemum leaves. In total, ten leaves were inoculated on two wound sites using a needle puncture technique for each isolate. The fungi from root and stem rot were inoculated on detached stems (15 cm length), and 5 stems (5 wound sites/stem) were used for each isolate. The inoculated leaves/stems were incubated in a moist plastic box until disease symptoms appeared. Then, the pathogens were re-isolated to fulfill Koch's postulates.

Pathogen identification using molecular technique

DNA extraction

Each fungal isolate was grown in potato dextrose broth for 2 days. Then, 120 mg of fungal mycelia were harvested for DNA extraction using the cetyltrimethylammonium bromide (CTAB) method. In brief, 600 µl of CTAB buffer was added to the mycelial tubes, ground, and incubated at 60ºC for 1 h. The mycelial tubes were centrifuged at 10,000 rpm for 10 min, after which, 600 µl of supernatant was collected. Then, 500 µl of chloroform: isoamyl alcohol (24:1) was added to the supernatant tube and mixed well. An equal volume of isopropanol and 0.1 volume of 3 M sodium acetate (pH 5.2) was added to the supernatant to precipitate fungal DNA. Then, the tube was centrifuged at 12,000 rpm for 10 min. The supernatant was removed and the DNA was washed with 200 µl of 70% ethanol and centrifuged at 12,000 rpm for 10 min. The supernatant was discarded, and the DNA pellet was dried for 1 h and then dissolved in nuclease-free water. The amount of genomic DNA was quantified using BioDrop (Biodrop Ltd., Cambridge, United Kingdom). The genomic DNA was kept at -20ºC until used.

Polymerase chain reaction (PCR) amplification and sequence alignment

Genomic DNA samples of the fungal isolates were confirmed using PCR amplification with the ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and

ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers. The PCR conditions for the internal transcribed spacer (ITS) procedure were 94ºC/5 min, followed by 35 cycles at 94ºC/1 min, 48ºC/1 min, and 72ºC/1 min, followed by 94ºC/5 min. The PCR products were visualized based on RedSafe™ (iNtRON Biotechnology, Inc., Gyeonggi-do, Korea) staining after gel electrophoresis in 1% agarose gel. Then, the PCR products were barcode taq sequenced by U2Bio (Thailand) Co., Ltd. The sequencing results were analyzed using Nucleotide BLAST on the NCBI database.

Fungicides and chemicals

This study investigated ten fungicides currently being used in chrysanthemum growing areas in northeast Thailand. Six of the fungicides could be categorized into four groups of single-site fungicides: quinone outside inhibitors (QoIs; azoxystrobin, pyraclostrobin and trifloxystrobin), demethylation inhibitors (DMIs; difenoconazole), dicarboximides (iprodione) and methyl benzimidazole carbamates (MBCs; thiophanate-methyl). The four others were multisite fungicides in the groups of chloronitriles (chlorothalonil), dithiocarbamates (mancozeb), and inorganic fungicides (copper oxychloride and sulfur). Salicylhydroxamic acid (SHAM, 99%; Alfa Aesar®; India), an alternative respiration inhibitor, was used for the sensitivity tests of the QoI fungicides (Avila-Adame *et al.,* 2003).

Fungicide sensitivity test of pathogens on culture medium

Agar plugs (each 0.5 cm in diameter) of the mycelia of pathogens aged 7 days and growing on PDA media were transferred to the centers of PDA plates supplemented with different fungicide concentrations of 0, 0.001, 0.01, 0.1, 1, 10, 100, and 1000 ppm. Sterile distilled water was added to the PDA medium for the control treatment. The control treatment of QoI fungicide testing was 100 ppm SHAM in a PDA medium. The culture plates were incubated at room temperature for 7 days. The colony size of the pathogens was measured in both vertical directions. The half maximal effective concentration (EC_{50}) value was calculated using probit analysis in the IBM SPSS Statistics 21.0 software. The EC_{50} value of each pathogen for each fungicide was classified into either sensitive or resistant isolates based on the fungicide baseline (Table 1).

Table 1. Sensitivity phenotypic classification of each fungicide to fungal pathogens causing leaf spots and wilt disease in chrysanthemums

Fungicide name and formulation	Phenotypic classification[1/]	
	Epicoccum and *Stemphylium*	*Fusarium*
Azoxystrobin 25% w/v SC Pyraclostrobin 10% w/v CS Trifloxystrobin 50% WG	S = EC_{50} <5 ppm, R = EC_{50} ≥5–100 ppm, HR = >100 ppm (Kreis *et al*., 2016 with modifications)	S = EC_{50} <1 ppm, MR = EC_{50} ≥1–10 ppm, R = EC_{50} >10 ppm (Andrade *et al*., 2022 with modifications)
Difenoconazole 25% w/v EC	S = EC_{50} <4 ppm, R = EC_{50} ≥4 ppm (Zhang *et al*., 2020 with modifications)	S = EC_{50} <5 ppm, R = EC_{50} ≥5 ppm (Rekanović *et al*., 2010 with modifications)
Iprodione 50% WP	S = EC_{50} <50 ppm, R = EC_{50} ≥50 ppm (Arora and Gopal, 2006 with modifications)	S = EC_{50} <10 ppm, R = EC_{50} ≥10 ppm (Gourlie and Hsiang, 2017 with modifications)
Thiophanate-methyl 70% WP	S = EC_{50} <10 ppm, MR = EC_{50} ≥10–100 ppm, R = EC_{50} >100-1000 ppm, HR = EC_{50} >1000 ppm (Vieira *et al*., 2017 with modifications)	S = EC_{50} <10 ppm, R = EC_{50} ≥10 ppm (Petkar *et al*., 2017)
Chlorothalonil 75% WP	S = EC_{50} ≤5 ppm, R = EC_{50} >5 ppm (Hollingshead, 2015)	S = EC_{50} ≤100 ppm, R = EC_{50} >100 ppm (Yamaguchi *et al*., 1998 with modifications)
Mancozeb 80% WP Copper oxychloride 77% WP Sulfur 80% WG	S = EC_{50} ≤100 ppm, R = EC_{50} >100 ppm (Malandrakis *et al*., 2015 with modifications)	

[1/] S = Sensitive, MR = Moderately resistant, R = Resistant, HR = Highly resistant

Effectiveness of fungicides for controlling leaf spot disease on detached leaf

Samples of chrysanthemum variety TISTR-NRCT01 aged 2 months and growing in 13 cm diameter pots were used in this experiment. The stems with healthy leaves were cut into 15 cm lengths and separately soaked in three different concentrations of each fungicide (5, 2.5 times reduced from the recommended dose, and the recommended dose). The fungicides, which were pathogen-sensitive, were used in this study. The leaf parts soaked in distilled water served as control. After drying, a leaf was punched with a needle puncture to make a wound. Then, a 0.5 cm diameter agar plug of a representative pathogen isolate was transferred to a wound site. The inoculated leaves were incubated in a moist plastic box under 12 h photoperiod at room temperature for 5 days. Three replicates were performed (five leaves in each replicate). The trial was repeated twice. The lesion size was measured based on two perpendicular lines. The percentage of disease control was calculated according to the formula as follows:

$$\text{Disease control (\%)} = \left(\frac{D1-D2}{D1}\right) \text{ x } 100$$

where, $D1$ is the mean of lesion size in the control treatment and $D2$ is the mean of lesion size in the fungicide treatment.

Effectiveness of fungicides for controlling leaf spot disease in pot conditions

In this experiment, the whole chrysanthemum plant in the pots mentioned above was sprayed with 50 ml of effective fungicide at the recommended dose for each fungicide. The plant sprayed with distilled water was used as a control. Then, the eight mature leaves were chosen on each plant and inoculated with a selected isolate of the pathogens using the mycelial agar plug. The inoculated plants were incubated in moist plastic bags for 24 h and kept indoors using a 16 h photoperiod at 25–28°C for 7 days. The experiment was repeated twice with three replicates. Then, the lesion size was measured. The percentage of disease control was calculated using a formula as above.

Statistical analysis

A completely randomized design was used for all experiments. The data were analyzed based on analysis of variance using the SPSS software version 25 (IBM Corp.; USA). Differences between means of treatments were examined using Duncan's multiple range test ($p < 0.05$). The lesion sizes of leaf spot diseases were expressed as mean ± standard deviation values.

Results

Pathogenicity test

Among fungal isolates, the most destructive on the detached chrysanthemum leaves and stems were KRS1 and LE4, respectively (Table 2). The colony characteristics of all isolates obtained from the artificially inoculated plant parts were identical to their initial ones (Figure 1).

Table 2. Disease severity on detached chrysanthemum leaves or stems after inoculation with different fungal isolates obtained from three provinces of Thailand, incubated for 7 days at room temperature

Province	Isolate name	Diseased part	Lesion size (cm)
Nakhon Ratchasima	KRS1	Leaf	0.92 ± 0.12[1/]
Ubon Ratchathani	UBO26	Leaf	0.38 ± 0.29[1/]
	UBO37	Root	0.71 ± 0.46[2/]
Loei	LE4	Stem	1.44 ± 0.30[2/]

[1/] Symptom occurring on leaves, [2/] Symptom occurring on stems, Values are mean ± standard deviation of 3 replicates

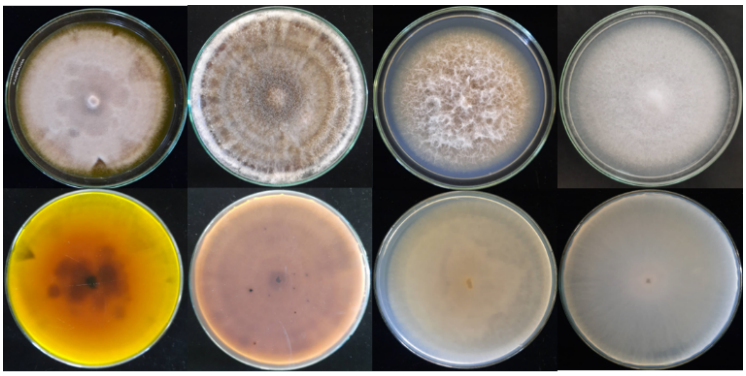

Figure 1. Morphological characteristics (upper row = obverse, lower row = reverse) of fungal colonies causing diseases on chrysanthemums at culture age 7 days, (A) UBO26 isolate, (B) KRS1 isolate, (C) UBO37 isolate, (D) LE4 isolate

Molecular pathogen identification

All isolates of the pathogens were analyzed based on the ITS region of the ITS5/ITS4 primers. The results indicated that two isolates of leaf spot pathogens (UBO26 and KRS1) were close to *Stemphylium lycopersici* and *Epicoccum sorghinum* with identities of 99.3% and 100%, respectively. Two isolates of wilt pathogens (UBO37 and LE4) showed similarity to *Fusarium solani* and *F. oxysporum* with identities of 99.16% and 99.81%, respectively (Figure 2). These four sequences of pathogen isolates were deposited in the GenBank database under accession numbers OQ979210, OQ979209, OQ979212, and OQ979207.

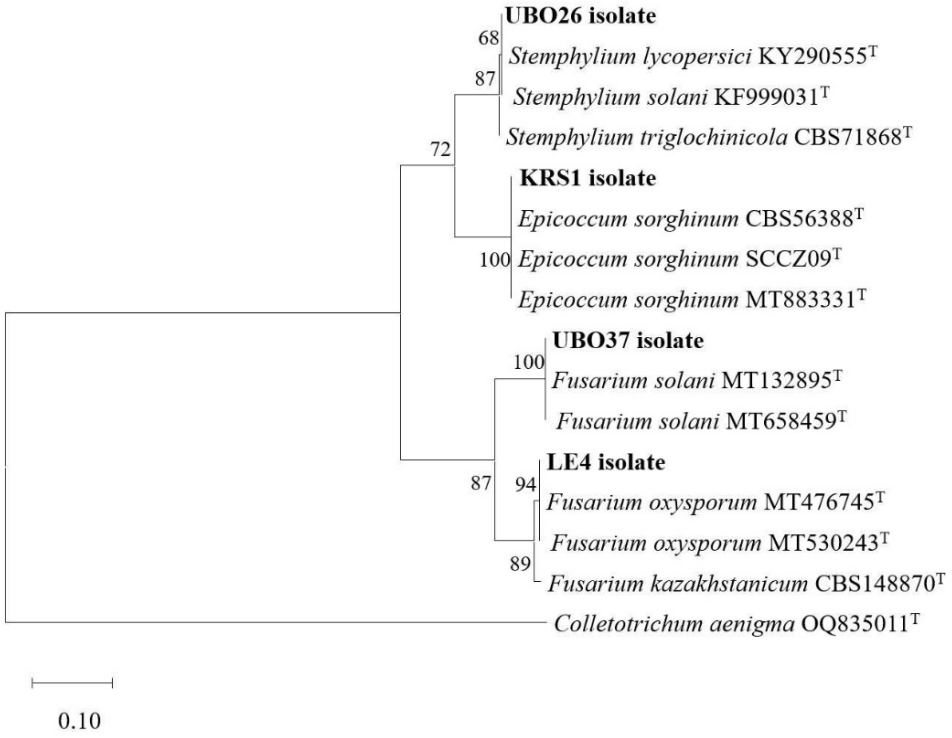

Figure 2. Maximum-likelihood phylogenetic tree based on ITS5 and ITS4 nucleotide sequences of four isolates of fungal chrysanthemum pathogens: UBO26 isolate compared with those of *Stemphylium* spp., KRS1 isolate compared with those of *Epicoccum sorghinum*, UBO37, and LE4 isolates compared with those of *Fusarium* spp., and with *Colletotrichum aenigma* as an out-group from the CBS and NCBI databases using MEGA X version Program (bootstrap of 1,000 replicates)

Fungicide sensitivity test of pathogens on culture medium

All pathogen isolates exhibited diverse resistant levels (MR, R, HR) to the tested fungicides for the fungicide sensitivity test. The leaf spot pathogen, *S. lycopersici* UBO26, was only sensitive to iprodione but insensitive to all tested QoI fungicides and to all multisite fungicides (chlorothalonil, mancozeb, copper oxychloride, and sulfur). *E. sorghinum* KRS1 was resistant to several chemicals except difenoconazole, iprodione and mancozeb (Table 3). The wilt pathogen, *F. solani* UBO37, was resistant to all the tested single-site fungicides and most of the multisite fungicides (mancozeb, copper oxychloride, and sulfur), fortunately, it was susceptible to chlorothalonil. Notably, *F. oxysporum* LE4 was sensitive to

one single-site fungicide (difenoconazole) and three multisite fungicides (copper oxychloride, chlorothalonil, and mancozeb), as shown in Table 3.

Table 3. Range of EC_{50} values of ten fungicides against four isolates of chrysanthemum pathogens and sensitivity phenotypic classification

Fungal pathogen	EC_{50} (ppm, a.i.)[1/]			
	<1–5	>5–10	>10–100	>100
S. lycopersici UBO26	iprodione (S)		difenoconazole (R)	azoxystrobin (HR) pyraclostrobin (HR) trifloxystrobin (HR) thiophanate-methyl (HR) chlorothalonil (R) mancozeb (R) copper oxychloride (R) sulfur (R)
E. sorghinum KRS1	difenoconazole (S) iprodione (S) mancozeb (S)	pyraclostrobin (R)	thiophanate-methyl (MR)	azoxystrobin (HR) trifloxystrobin (HR) chlorothalonil (R) copper oxychloride (R) sulfur (R)
F. solani UBO37		difenoconazole (R)	chlorothalonil (S) thiophanate-methyl (R)	azoxystrobin (R) pyraclostrobin (R) trifloxystrobin (R) iprodione (R) mancozeb (R) copper oxychloride (R) sulfur (R)
F. oxysporum LE4	difenoconazole (S)	copper oxychloride (S)	chlorothalonil (S) mancozeb (S) thiophanate-methyl (R)	azoxystrobin (R) pyraclostrobin (R) trifloxystrobin (R) iprodione (R) sulfur (R)

[1/]S = Sensitive, MR = Moderately resistant, R = Resistant, HR = Highly resistant

Effectiveness of fungicides for controlling leaf spot disease on detached leaf

Notably, almost all concentrations of the three fungicides—difenoconazole (400 and 1,000 ppm), iprodione (600 and 1,500 ppm), and mancozeb (800 and 2,000 ppm)—significantly reduced the severity of the leaf spot disease caused by *E. sorghinum* KRS1. Also notable was that the recommended dose of two fungicides—difenoconazole (1,000 ppm) and mancozeb (2,000 ppm)—greatly controlled the disease by 76.9 and 84.6%, respectively (Table 4 and Figure 3).

Table 4. Lesion size and percentage of leaf spot disease control on chrysanthemum detached leaves after fungicide soaking in diverse concentrations of difenoconazole, iprodione, and mancozeb, followed by inoculation with *Epicoccum sorghinum* KRS1, incubated for 5 days after inoculation

Fungicide	Concentration (ppm a.i.)	Lesion size (cm)	Disease control (%)
Water (Control)	0	1.30 ± 0.265^{d}	–
Difenoconazole	200	1.42 ± 0.074^{e}	-9.2
	400	0.56 ± 0.055^{b}	56.9
	1,000	0.30 ± 0.067^{a}	76.9
Iprodione	300	1.15 ± 0.131^{cd}	11.5
	600	1.00 ± 0.133^{c}	23.1
	1,500	0.76 ± 0.127^{b}	41.5
Mancozeb	400	1.15 ± 0.070^{cd}	11.5
	800	0.64 ± 0.099^{b}	50.8
	2,000	0.20 ± 0.076^{a}	84.6

Mean values followed by the same lowercase superscript are not significantly different according to Duncan's multiple range test ($p< 0.05$). Values are mean ± standard deviation of 3 replicates.

Effectiveness of fungicides for controlling leaf spot disease in pot conditions

The recommended concentrations of difenoconazole, iprodione, and mancozeb, significantly suppressed the severity of disease in the pots by 28.5–93.9%. Mancozeb was the most effective fungicide in controlling the disease (93.9% reduction), followed by difenoconazole (60%), as shown in Table 5 and Figure 4.

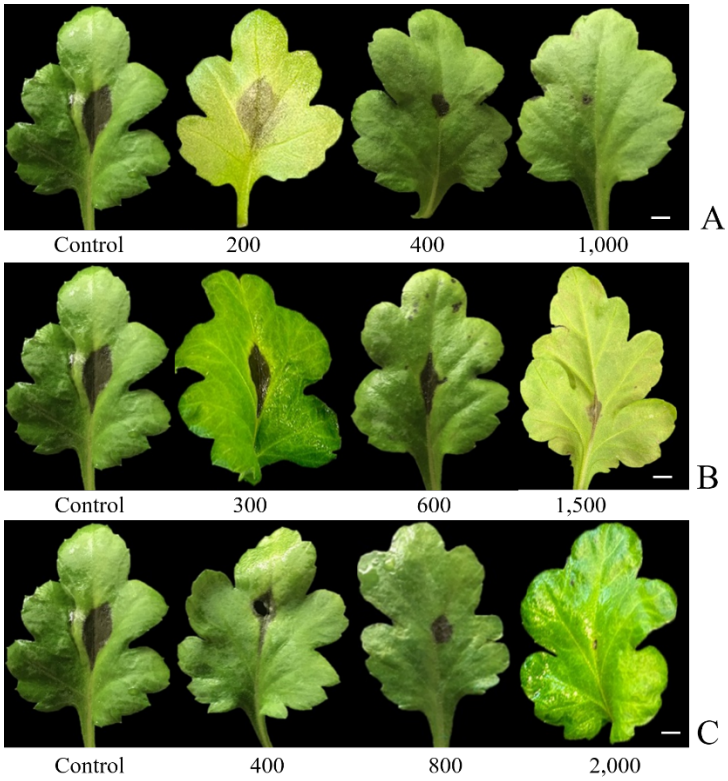

Figure 3. Representative chrysanthemum leaf spot symptoms at 5 days of inoculation with *Epicoccum sorghinum* KRS1 on detached leaves after soaking in (A) difenoconazole at 200, 400, and 1,000 ppm, (B) iprodione at 300, 600, and 1,500 ppm, (C) mancozeb at 400, 800, and 2,000 ppm, scale bar = 5 mm

Table 5. Lesion size and percentage of leaf spot disease control of chrysanthemum in pot experiment after fungicide spraying at the recommended dose followed by inoculation with *Epicoccum sorghinum* KRS1, incubated for 7 days after inoculation

Fungicide	Recommended concentration (ppm a.i.)	Lesion size (cm)	Disease control (%)
Water (Control)	0	1.65±0.121^{d}	–
Difenoconazole	1,000	1.05±0.076^{b}	60.0
Iprodione	1,500	1.18±0.080^{c}	28.5
Mancozeb	2,000	0.10±0.020^{a}	93.9

Mean values followed by the same lowercase superscript are not significantly different according to Duncan's multiple range test ($p< 0.05$). Values are mean ± standard deviation of 3 replicates

Figure 4. Representative chrysanthemum leaf spot symptoms at 7 days after inoculation with *Epicoccum sorghinum* KRS1 from potted plants after spraying with difenoconazole, iprodione, and mancozeb at the recommended dose for each fungicide, scale bar = 10 mm

Discussion

In this study, *E. sorghinum* was the dominant causal agent of leaf spot disease in chrysanthemum crops from the fields sampled in Nakhon Ratchasima province, in accordance with the findings of Chen *et al*. (2021) showing that *E. sorghinum* caused leaf spot disease in chrysanthemums in China. Likewise, in Ubon Ratchathani province, *S. lycopersici* was currently found as a leaf spot causative agent on chrysanthemums. In Japan, Nishi *et al*. (2009) reported that *S. lycopersici* was the pathogen of ray specks on chrysanthemums. In the current study, *E. sorghinum* displayed more virulence than *S. lycopersici*. In Thailand, leaf spot occurrences have frequently been accompanied by wilt disease in chrysanthemum fields. In the current trial, root and stem rot pathogens from Ubon Ratchathani and Loei provinces were molecularly identified as *F. solani* and *F. oxysporum,* respectively. *Fusarium* spp. is a serious soilborne pathogen and causes wilt disease in chrysanthemums in many countries (Kwon *et al*., 2013; Singh and Kumar, 2014; Thao *et al*., 2021).

According to the fungicide sensitivity test on chrysanthemum pathogens, the various fungicides used in the current study displayed resistance to the pathogens. Only a few fungicides were sensitive to pathogens, such as dicarboximide iprodione, which is sensitive to *S. lycopersici* UBO26. This result was similar to Gálvez *et al*. (2016), who reported that *S. vesicarium*, a garlic leaf blight pathogen, was highly sensitive to iprodione and the DMI fungicide prochloraz. Additionally, most isolates of *S. solani* causing tomato leaf spots

were susceptible to SDHI fungicide fluxapyroxad (Bi *et al*., 2022). *E. sorghinum* KRS1 was susceptible to difenoconazole, iprodione and mancozeb. Similar to Yao *et al*. (2023) highlighted that *E. sorghinum*, causing hydrangea leaf spots, expressed high sensitivity to the DMI fungicide difenoconazole, while Taguiam *et al*. (2020) showed that another isolate of *E. sorghinum*, causing dragon fruit stem rots, was sensitive to mancozeb. In addition, Xu *et al*. (2022) demonstrated that *Epicoccum* species causing corn leaf spots are susceptible to the MBC fungicide carbendazim and the DMI fungicide tebuconazole. The results from the current study indicated that *F. solani* UBO37 and *F. oxysporum* LE4 were sensitive to multisite chlorothalonil. Only the latter fungus was susceptible to a single-site fungicide (the DMI fungicide difenoconazole) and two multisite fungicides (copper oxychloride and mancozeb). Similarly, it has been reported that *Fusarium* isolates were sensitive to the DMI fungicides difenoconazole, tebuconazole and imazalil (Gachango *et al*., 2012; Frąc *et al*., 2016; Maniçoba *et al*., 2023), as well as to the MBC fungicide thiabendazole (Gachango *et al*., 2012). However, the sensitivity to multisite fungicides of the genus *Fusarium* was established based on the current results.

The study of fungicide resistance on pathogens of chrysanthemum has been rarely documented. Some of the publications stated that *P. horiana*, the white rust causal agent, was particularly resistant to QoI via gene mutation at cytochrome *b* N256 (Matsuzaki *et al*., 2021b) and DMI as well as multisite fungicides (Ishii, 2006; Palmer *et al*., 2015).

The resistance of *Stemphylium* has been reported to several fungicides. Hay *et al*. (2019), Stricker *et al*. (2021) and Wang *et al*. (2021) reported that *S. vesicarium*, causing leaf blight on onions, was resistant to the QoI fungicide azoxystrobin. Alberoni *et al*. (2005) and Alberoni *et al*. (2010) showed that isolates of *S. vesicarium*, causing brown spots on pears, were resistant to both QoI fungicides, such as kresoximmethyl, trifloxystrobin, as well as pyraclostrobin and dicarboximide fungicides, such as procymidone and iprodione. Lin and Fan (2023) demonstrated that *S. solani*, causing leaf spots on tomatoes, was resistant to SDHI fungicides, such as boscalid, and QoI fungicides, such as pyraclostrobin. The point mutation of G143A at the *cytochrome b* gene conferred fungicide-resistant ability to *S. vesicarium* (Hay *et al*., 2019).

E. sorghinum was also resistant to many kinds of tested fungicides used in the current investigation. However, there is a lack of documented information on the fungicide resistance of the genera *Epicoccum*. The current results represent the first record regarding *Epicoccum* resistance to fungicides.

Fusarium spp. was also resistant to many fungicides used in the current experiment. The fungicide resistance of wilt chrysanthemum pathogens, including *Fusarium*, has been rarely studied but has been reported in other plant

species. For example, isolates of *F. circinatum* on pines were insensitive to the QoI fungicide pyraclostrobin and the Phenylpyrrole fungicide fludioxonil (Mullett *et al*., 2017). In addition, several species of *Fusarium* on wheat were highly resistant to the QoI fungicides azoxystrobin and fluoxastrobin (Müllenborn *et al*., 2008). Other factors besides a point mutation of the *cytochrome b* gene have been identified as mutation mechanisms of *Fusarium* resistance to QoI fungicides (Andrade *et al*., 2022). *F. pseudograminearum*, with two site mutations at the *SdhC1* gene, expressed resistance to the SDHI fungicide pydiflumetofen (Li *et al*., 2023).

The effective fungicides in controlling *Epicoccum* leaf spots on detached leaves were the DMI fungicide difenoconazole and the multisite fungicide mancozeb. The DMIs provide good control of diverse fungal diseases with the mode of action of a sterol biosynthesis inhibitor in fungal cell membranes (Ishii and Holloman, 2015; Ishii *et al*., 2021). Their resistance mechanism is involved in the mutation at the target site in the *CYP51* gene (Zhang *et al*., 2020; Ishii *et al*., 2021; Spanner *et al*., 2021).

Even more notable was that a multisite fungicide, mancozeb, could control the disease better than the two systemic fungicides, difenoconazole and iprodione, for the pot conditions. Mancozeb has been documented as an effective fungicide controlling several plant diseases (Liu *et al*., 2021; Aggarwal *et al*., 2023; Rashid *et al*., 2023). Specifically, Liu *et al*. (2021) noted that applying mancozeb prior to pathogen inoculation could completely control the disease. Based on the current results, mancozeb could reduce disease by 93.9%. Furthermore, the current trial was performed indoors, so less chemical dissipation may be a reasonable assumption. The mancozeb mechanism is a multisite inhibitor with low-risk resistance development. It could be used in rotation or in combination with single-site fungicides to enhance chrysanthemum disease management in some areas. Indeed, it needs to be monitored frequently for resistant development, since this investigation exposed mancozeb-resistant strains in Ubon Ratchathani province.

Acknowledgements

The authors thank Kamonwan Sichai and Warisa Srisopha for their help in pathogen identification using molecular techniques. This study was financially supported by the National Research Council of Thailand (Grant number: N21A650695).

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(Received: 10 October 2024, Revised: 28 May 2025, Accepted: 1 July 2025)