Baseline Sensitivity and Action Mechanism of Propamidine Against *Alternaria brassicicola*, the Causal Agent of Dark Leaf Spot on Cabbage

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Abstract

In the current study, a total of 53 isolates of *Alternaria brassicicola* collected from Shaanxi Province of China were characterized for their sensitivity to propamidine. The EC₅₀ (50% effective concentration) values for propamidine inhibiting mycelial growth and spore germination ranged from 0.515 to 3.247 µg/ml and 0.393 to 2.982 µg/ml, with average EC₅₀ values of 1.327 ± 0.198 µg/ml and 1.106 ± 0.113 µg/ml, respectively. In greenhouse experiments, propamidine at 100 µg/ml provided >90% efficacy against dark leaf spot on cabbage, which was higher than the efficacy obtained by azoxystrobin at the same concentration. After treatment with propamidine, fungal growth distortions were observed in the form of excess mycelial branching, thickened cell walls, decreased cell membrane permeability, and increased chitin content.

Interestingly, colony color faded after treatment with propamidine compared with that of the untreated parental isolates. Importantly, the expressions of melanin biosynthesis-associated genes *Amr1*, *Scd1*, *Brn1*, and *Brn2* were downregulated at different levels. The obtained baseline sensitivity and control efficacy data suggested that propamidine inhibited not only growth of *A. brassicicola* but also melanin biosynthesis, which could reduce the biocompatibility of *A. brassicicola* in the field. These biological characteristics encourage further investigation of the mechanism of action of propamidine against *A. brassicicola*.

Keywords: propamidine, *Alternaria brassicicola*, baseline sensitivity, action mechanism

Cabbage (*Brassica oleracea* var *capitata* L.) is one of the most popular and widely grown vegetables worldwide, especially in China, and it accounts for 40% of global production (Ayaz et al. 2006; Zhang et al. 2007). Dark leaf spot, a disease caused by the filamentous ascomycete *Alternaria brassicicola* (Schwein). Wiltshire, is one of the most devastating diseases of cabbage (Cho et al. 2012). All developmental phases of cabbage may be infected by *A. brassicicola*, from seedling to maturity (Kim et al. 2007). This disease occurs throughout major cabbage-growing regions worldwide, including China, Japan, Canada, India, and the United States. Yield losses up to 70% caused by the disease have been reported (Kreis et al. 2016; Chen et al. 2017; Nowakowska et al. 2019; Srivastava et al. 2012). Conidia produced by *A. brassicicola* are dispersed by wind and rain splash, and the disease is difficult to manage (Manhas and Kaur 2016).

The use of resistant cultivars would be an ideal method to control the disease, but crucifer cultivars resistant to dark leaf spot are currently unavailable (Pedras et al. 2009). Consequently, chemical control is still the most reliable and cost-effective way of reducing disease incidence. Fungicides with differing modes of action commonly used in the management of dark leaf spot include benzimidazoles (carbendazim), dicarboximides (iprodione), phenylpyrroles (fludioxonil), strobilurins (azoxystrobin), and dithiocarbamates (mancozeb) (Iacomini-Vasilescu et al. 2004; Manhas and Kaur 2016). To date, there have been no reported control failures for fungicides used to manage *A. brassicicola*; however, resistance to these fungicide groups has been widely reported in many other plant pathogens. For example, resistance to carbendazim has been reported for *Sclerotinia sclerotiorum*, *Botrytis cinerea*, and *Fusarium*

graminearum (Beever et al. 1989; Chen and Zhou 2009; Xu et al. 2015); resistance to iprodione and fludioxonil has been reported for *A. brassicicola* (Iacomini-Vasilescu et al. 2004); resistance to azoxystrobin has been reported for >30 pathogen species, such as *Alternaria alternata*, *B. cinerea*, and *Plasmopara viticola* (Bartlett et al. 2002; Köller 2002); and resistance to mancozeb has been detected for *Fulvia fulva*, *Colletotrichum gloeosporioides*, and *A. alternata* (Gullino et al. 2010; Malandrakis et al. 2015; Wang et al. 2005; Yang et al. 2013). Considering the potential fungicide resistance risk with *A. brassicicola*, it is necessary to screen new alternative fungicides for disease management.

Propamidine belongs to a novel chemical class of aromatic diamidine compounds, which include other compounds such as betamidine, butamidine, and pentamidine (Hou et al. 2010). The antifungal activity of propamidine against agricultural fungi was first documented against *B. cinerea*, a causal agent of gray mold. Subsequent research found that propamidine also exhibited high efficacy against other fungal diseases, including *Sclerotinia* stem rot caused by *S. sclerotiorum* and apple valsa canker caused by *Valsa mali* Miyabe & Yamada (Wang et al. 2017, 2018). However, the mechanism of action of propamidine is still unclear. In China, propamidine has been registered since 2004, and only for the control of gray mold on vegetables.

There is little information about the sensitivity and resistance risk of *A. brassicicola* to propamidine. Such a study would provide an opportunity to study the antifungal activity and mechanism of action of propamidine against *A. brassicicola* prior to it being registered for use to manage dark leaf spot on cabbage in China.

The aims of the present study were to: (i) establish the baseline sensitivity of *A. brassicicola* to propamidine using inhibition of mycelial growth and spore germination data; (ii) evaluate the efficacy of propamidine against dark leaf spot on cabbage in greenhouse experiments; (iii) investigate the effect of propamidine on the morphology of *A. brassicicola*; and (iv) explore the mechanism of action of propamidine against *A. brassicicola*.

Materials and Methods

Isolates, media, and fungicides. A total of 53 *A. brassicicola* isolates were collected from naturally infected cabbages in Shaanxi Province during 2017 and 2018. All isolates had been exposed to carbendazim, a benzimidazole fungicide. Isolates were confirmed by microscopic observations of morphological characteristics and purified by three rounds of single-spore isolation.

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Potato dextrose agar (PDA; 200 g of potato, 16 g of agar, and 20 g of dextrose per liter of distilled water) was used for the mycelial sensitivity test, and water agar (WA; 20 g of agar power per liter of distilled water) was used in the spore germination test.

Technical grade propamidine (97.0%) provided by the Research and Development Center of Biorational Pesticide of Northwest A & F University and azoxystrobin (97.8%; Syngenta China Co., Ltd., China) were dissolved in methanol at 10 mg/ml as stock solutions.

Baseline sensitivity of *A. brassicicola* to propamidine. Mycelial plugs (diameter, 5 mm) taken from the edge of a 6-day-old colony of each *A. brassicicola* isolate were transferred to a series of PDA plates containing 0, 0.625, 1.25, 2.5, 5, or 10 µg/ml of propamidine. After incubation at 25°C in darkness for 6 days, colony diameters were measured at right angles, and the effective concentration at which the mycelial growth rate was inhibited by 50% (EC₅₀) was calculated according to probit analysis (Ma et al. 2009). The experiment was performed twice with three replicates per concentration.

The effects of propamidine on spore germination of *A. brassicicola* isolates were measured according to the method described in previous studies with minor modifications (Manhas and Kaur 2016; Mc 2010; Zhang et al. 2014). Spore suspensions (1×10^5 ml⁻¹) for each isolate were prepared in sterile distilled water from 6-day-old colonies. The suspensions were then transferred to WA plates containing 0, 0.625, 1.25, 2.5, 5, or 10 µg/ml of propamidine. After incubation at 25°C in darkness for 24 h, the percentage of spore germination (200 spores for each treatment) was observed under a microscope using a micrometer. A spore was scored as germinated when the germ tube length was at least half the conidium length, and the EC₅₀ was calculated. The experiment was performed twice with three replicates per treatment.

Efficacy of propamidine against *A. brassicicola*. The efficacy of propamidine against *A. brassicicola* was assayed in pot experiments. Cabbage plants (Zhong gan 11) with four to six leaves were sprayed until run-off with propamidine at concentrations of 25, 50, or 100 µg/ml and azoxystrobin (reference fungicide) at 100 µg/ml. Plants sprayed with water were used as a control. Plants were left to dry naturally; then, leaves were wounded with a sterilized needle (diameter, 0.5 mm) and inoculated with mycelia plugs (one plug per leaf and three leaves per plant) cut from the margin of 6-day-old colonies of the *A. brassicicola* isolates YL12 and RG5 (randomly selected). All plants were placed in a growth chamber at 20 to 25°C with a 12-h photoperiod and 80% RH. After 6 days, the diameter of each lesion was measured and the control efficacy (the reduced severity relative to the control) was calculated as follows:

$$\text{Control efficacy} = \frac{(\text{lesion diameter of control} - \text{lesion diameter of treatment})}{(\text{lesion diameter of control} - 0.5)} \times 100$$

The experiments were performed twice with six plants per fungicide treatment.

Effect of propamidine on mycelial and colony morphology. Mycelial plugs (diameter, 5 mm) cut from the leading edge of actively growing colonies of isolates YL12 and RG5 were transferred to PDA plates amended with propamidine at their respective EC₅₀ values. PDA plates without propamidine were used as the control. After 72 h of culture at 25°C in a growth chamber, the margin of the colony (10 mm × 10 mm) was transferred to slide glass. The morphology of the mycelial surface was observed with a scanning electron microscope (SEM; JSM-6360LV, Japan) and transmission electron microscope (TEM; HITACHI Transmission Electron Microscope HT7700, Japan) (Wang et al. 2017).

To test the effect of propamidine on colony morphology, mycelial plugs were transferred to PDA plates treated with propamidine at their EC₁₀ and EC₂₀ values. After incubation at 25°C in darkness for 1 week, the colonies were photographed. The experiments were performed twice with three replicates per treatment.

Effect of propamidine on cell membrane permeability. Two *A. brassicicola* isolates, YL12 and RG5, were used in this experiment. Ten mycelial plugs taken from the edge of a 6-day-old colony were transferred to 250-ml flasks containing 100 ml of potato dextrose broth

(PDB; PDA without agar). After incubation on a shaker set at 175 rpm, 25°C, and in darkness for 4 days, three flasks of each treatment were supplemented with propamidine at their respective EC₅₀ values. After shaking for an additional 24 h, mycelia were collected, strained through three layers of filter paper, and washed with distilled water three times. Then, 0.2 g of mycelia per sample was suspended in 20 ml of distilled water, and the electrical conductivity of the distilled water was measured after 0.5, 1, 2, 3, 4, and 5 h with an electrical conductivity meter (S320 Sevencompact; Mettler Toledo, Switzerland). After 5 h, each sample was boiled for 5 min, and the final conductivity was determined. The relative conductivity was calculated as follows (Zhang et al. 2018):

$$\text{relative conductivity} = \text{conductivity/final conductivity} \times 100$$

The experiment was conducted three times with three flasks per treatment.

Effect of propamidine on chitin content. Two isolates, YL12 and RG5, were used for the chitin (*N*-acetylglucosamine, GlcNAc) content test using the Morgan-Elson method according to previous studies, with some modifications (Costa-de-Oliveira et al. 2013; Fortwendel et al. 2009; Walker et al. 2008). Briefly, 5-mm mycelial plugs cultured in flasks of PDB were treated with propamidine at their respective EC₅₀ values and collected as described. For each treatment, 5 mg of dry mycelia was suspended in 1 ml of 6% KOH and heated at 80°C for 90 min. Samples were centrifuged at 12,000 rpm for 10 min, and the supernatant was discarded. The precipitates were resuspended in 1 ml of PBS in three cycles of centrifugation and resuspension (12,000 rpm, 10 min). The precipitates were finally resuspended in 0.5 ml of McIlvaine's buffer (pH 6.0), and 100 µl (5 mg/ml in phosphate-buffered saline) of *Streptomyces plicatus* chitinase (Sigma, USA) was added to hydrolyze chitin to GlcNAc. After the samples were incubated at 37°C for 24 h with gentle mixing, 100 µl of each sample was combined with 100 µl of 0.27 M Mosadium borate (pH 9.0) in a 1.5-ml centrifuge tube and heated at 100°C for 10 min. Then, 1 ml of freshly diluted (1:10) Ehrlich's reagent (10 g *p*-dimethylaminobenzaldehyde in 12.5 ml of concentrated HCl and 87.5 ml glacial acetic acid) was added. After incubating at 37°C for 20 min, the absorbance of the sample at 585 nm was recorded. Standard curves were established from stocks of 0.2 to 2.0 mM GlcNAc (Sigma, USA) (Zhang et al. 2010). The experiment was repeated three times with three flasks per treatment.

Effect of propamidine on the expression of genes associated with melanin biosynthesis. Previous studies have demonstrated that the *Alternaria* melanin regulation gene *Amr1* (GenBank accession number in NCBI: JF487829), scytalone dehydratase gene *Scd1* (Ab03393.1), 1,3,8-trihydroxynaphthalene (T3HN) gene *Brn1* (Ab02276.1), and 1,3,6,8-tetrahydroxynaphthalene (T4HN) gene *Brn2* (Ab02275.1) were involved in melanin biosynthesis (Cho et al. 2012; Tsuji et al. 2002). Therefore, real-time quantitative reverse-transcription PCR (qRT-PCR) was used to test the expression levels of these genes in response to propamidine exposure. Two *A. brassicicola* isolates, YL12 and RG5, were used in this test, and the experiment was conducted as follows: 10 mycelial plugs cut from the margin of each 6-day-old colony were transferred to 250-ml flasks containing 100 ml of PDB. After shake incubation at 175 rpm and 25°C for 4 days, some flasks were supplemented with propamidine at their respective EC₅₀ values. After shaking for an additional 24 h, mycelia were collected. Total RNA was extracted using the TRIzol Reagent (Invitrogen). The extracted RNA was treated with DNase and used for cDNA synthesis with a PrimeScript RT reagent kit (TaKaRa). The qRT-PCR amplifications were performed using a CFX96 real-time detection system (Bio-Rad, Hercules, CA) using SYBR Premix Ex Taq II (Takara, Dalian). Using the genome sequence of *A. brassicicola* (<https://jgi.doe.gov/Abbrassicicola>), the primers listed in Table 1 were designed according to a previous study (Cho et al. 2012). Relative expression levels of each gene were calculated using the 2^{-ΔΔC_t} method (Livak and Schmittgen 2001). Each treatment had three technical replicates, and the experiment was conducted with three biological replicates.

Data analysis. All data obtained were analyzed using an analysis of variance (ANOVA) with SPSS 22.0 (SPSS Inc., Chicago, IL). A significant difference analysis of the data of EC₅₀ values was

performed and the biological characteristics were determined using Fisher's least significant difference (LSD) test at 0.05 levels.

Results

Baseline sensitivity of *A. brassicicola* to propamidine. None of the 53 *A. brassicicola* isolates used in this study had been exposed to propamidine. The EC₅₀ values of each isolate were calculated by regressing the percentage growth inhibition against the log-transformed fungicide concentration (Ma et al. 2009). The mycelial growth inhibition assays indicated that the EC₅₀ values for propamidine ranged from 0.515 to 3.247 µg/ml, with a mean of 1.327 µg/ml ± 0.198 µg/ml. The spore germination inhibition assays indicated that the EC₅₀ values ranged from 0.393 to 2.982 µg/ml, with a mean of 1.106 ± 0.113 µg/ml. The frequency distribution of the EC₅₀ values for both mycelial growth and spore germination were unimodal with a narrow range (Fig. 1), and the variation factor values (VF; the maximum EC₅₀ value/the minimum EC₅₀ value) were 6.30 and 7.59, respectively.

Efficacy of propamidine against *A. brassicicola*. On cabbage leaves, large lesions developed on control plants exposed to *A.*

brassicicola isolates YL12 and RG5 when sprayed with only water. The control efficacy of propamidine against *A. brassicicola* on potted cabbage plants at 100 µg/ml provided 90.7% efficacy, which was better compared to the efficacy obtained by the reference fungicide azoxystrobin at the same concentration. When propamidine was sprayed at 50 µg/ml, 65.3% control efficacy was observed. Moreover, nearly

Table 2. Efficacy of propamidine against *A. brassicicola* in pot experiments^y

Treatment	Lesion diameter (cm)	Control efficacy (%) ^z
Propamidine (25 µg/ml)	2.37 b	39.87 c
Propamidine (50 µg/ml)	1.58 c	65.27 b
Propamidine (100 µg/ml)	0.79 d	90.68 a
Azoxystrobin (100 µg/ml)	0.92 d	86.50 a
Water	3.61 a	—

^y Data are the means of two isolates, YL12 and RG5. Mean values with the same letters within the same column were not significantly different (*P* = 0.05, Fisher's least significant difference).

^z Control efficacy = [(Lesion diameter of control – Lesion diameter of treated group)/(Lesion diameter of control – 0.5)] × 100%.

Table 1. Primers used in this study

Primer	Sequence (5'-3')	Use ^z
P1	CACCCAAAGCTTCCTCCATA	qRT-PCR primers for the <i>Amr1</i> gene
P2	CAGGCGGAAGAAGAACAAG	
P3	GAGGACGAGATTGTCGGGTA	qRT-PCR primers for the <i>Scd1</i> gene
P4	ACTCGAACCACCTGATGTCC	
P5	GCGAGTACATTCTGGTGGT	qRT-PCR primers for the <i>Brn1</i> gene
P6	GTTGACCCAGTCACCGTCTT	
P7	GGACGTAACGGAGGAGATCA	qRT-PCR primers for the <i>Brn2</i> gene
P8	TCTTCGATGTGCTTGTACGC	
actin-F	GGCAACATTGTCATGTCTGG	qRT-PCR primers for analysis of actin gene expression
actin-R	GAGCGAAGCAAGAATGGAAC	

^z qRT-PCR, quantitative reverse-transcription PCR.

Fig. 1. Frequency distribution of propamidine EC₅₀ values for 53 isolates of *A. brassicicola* by inhibiting mycelial growth and spore germination.

40% control efficacy was observed when treated with propamidine at 25 $\mu\text{g/ml}$ (Table 2).

Effect of propamidine on mycelial and colony morphology.

The SEM observations showed that the mycelia of *A. brassicicola* were severely distorted, with a brush-like cluster of hyphae at the hyphal tip on PDA supplemented with propamidine at their EC_{50} values (Fig. 2A; Supplementary Fig. S1). Observations using TEM showed that the mycelial cell wall of *A. brassicicola* was thicker than that of

the untreated control (Fig. 2B; Supplementary Table S1). In addition, the colonies of both *A. brassicicola* isolates YL12 and RG5 were lighter in color when grown on PDA amended with propamidine at their respective EC_{10} or EC_{20} values than when grown on PDA with no amendments (Fig. 3).

Effect of propamidine on cell membrane permeability. The relative conductivity of the mycelia of *A. brassicicola* isolates YL12 and RG5 treated with or without propamidine increased over time.

Fig. 2. Effect of propamidine on mycelial morphology of two wild-type *A. brassicicola* isolates (YL12 and RG5). **Top four images:** Scanning electron microscope (SEM) observations. **Bottom four images:** Transmission electron microscope (TEM) observations.

Additionally, the relative conductivity of the mycelia treated with propamidine was always lower than that of the control (Fig. 4).

Effect of propamidine on chitin content. As shown in Figure 5, the natural chitin content of the mycelia of the two tested isolates differed from each other. When treated with propamidine at their EC_{50} values, however, the chitin content of *A. brassicicola* isolates YL12 and RG5 increased by 97 and 134%, respectively.

Expression of melanin biosynthesis-associated genes. For both isolates examined, the expression levels of the *Amr1*, *Scd1*, *Brn1*, and *Brn2* genes involved in the melanin synthesis pathway differed from that of their respective controls. The expression levels of the four genes in YL12 and RG5 was downregulated after treatment with propamidine, especially the *Amr1* gene, which exhibited downregulated expression of >60% (Fig. 6).

Discussion

Propamidine belongs to the chemical class of aromatic diamidine compounds. Currently, propamidine has been registered in China for the control of gray mold caused by *B. cinerea* on tomato and cucumber, but it has not been registered for the control of dark leaf spot on cabbage (Hou et al. 2010). In this study, propamidine sensitivity, as indicated by EC_{50} values, was determined for 53 isolates of *A. brassicicola* according to mycelial growth and spore germination inhibition. The average EC_{50} values of the 53 isolates for inhibiting mycelial growth and spore germination were $1.327 \mu\text{g/ml} \pm 0.198 \mu\text{g/ml}$ and $1.106 \pm 0.113 \mu\text{g/ml}$, respectively. These values were lower than the average propamidine EC_{50} value ($1.616 \pm 0.217 \mu\text{g/ml}$) reported for *S. sclerotiorum* (Wang et al. 2017), and lower than that of azoxystrobin against *A. brassicicola* (mean EC_{50} value $2.49 \mu\text{g/ml}$) (Kreis et al. 2016). These low EC_{50} values and the narrow range of frequency distribution indicated that no resistant subpopulations were detected among the isolates used in this study. These sensitivity data could be used as a relative baseline for future monitoring of the shift of sensitivity in *A. brassicicola* populations

to propamidine. Additional isolate collection from different locations and sensitivity tests are underway in our laboratory.

Pot experiments indicated that >90% efficacy was observed when propamidine was applied at $100 \mu\text{g/ml}$, which was higher than the efficacy of the reference fungicide azoxystrobin at the same concentration. These results suggested that propamidine exhibits potential as an alternative candidate for the control of dark leaf spot on cabbage.

Chitin, a linear and insoluble homopolymer composed of β -1,4-linked subunits of the acetylated amino sugar N-acetylglucosamine, is an essential structural component of the cell walls of filamentous fungi. It is the second most abundant polymer found in the biosphere (Seidl 2008). In this study, we observed that the mycelial cell wall of *A. brassicicola* isolates became thicker after treatment with propamidine, which was consistent with the results of treating *S. sclerotiorum* with propamidine (Wang et al. 2017). Moreover, the chitin content in *A. brassicicola* increased significantly after treatment with propamidine, which may be considered a protective measure from the damaging effects of the fungicide; this finding was consistent with the increased thickness of the mycelial cell wall of *A. brassicicola*. In addition, the mycelia were distorted, with more branches, and the relative conductivity was reduced after treatment with propamidine, indicating that propamidine could reduce the cell membrane permeability of *A. brassicicola*. This phenomenon was consistent with the results of treating *S. sclerotiorum* with prochloraz (Zhang et al. 2018).

Melanin, a dark brown or black pigment tissue produced by *Alternaria* species, is a ubiquitous pigment that has an important role in protecting fungi from the damaging effects of environmental stress; therefore, it may be considered an advantageous adaptation (Eliahu et al. 2007). Previous studies have demonstrated that melanin could enhance the tolerance of fungi to ultraviolet (UV) light, enzymatic lysis, and extreme temperatures (Rehnstrom and Free 1996; Rosas and Casadevall 1997; Wang and Casadevall 1994). In contrast, melanin-deficient mutants were more sensitive to UV light, and the conidial

Fig. 3. Effect of different concentrations of propamidine (EC_{10} and EC_{20} value) on colony morphology of two wild-type *A. brassicicola* isolates (YL12 and RG5). **A**, Untreated control. **B**, Propamidine at EC_{10} value. **C**, Propamidine at EC_{20} value.

size and septal number were reduced in *A. alternata* (Kawamura et al. 1999). Melanin is also required for the mechanical penetration of host plants by other phytopathogenic fungi, such as *Magnaporthe grisea* and *Colletotrichum lagenarium* (Kubo et al. 1991). Melanin-deficient mutants of *Cochliobolus heterostrophus* lack pathogenicity under field conditions (Oide et al. 2006). Because of the lack of melanin standards, the melanin content was only qualitative in the present study. As shown in Figure 3, the black coloration of the *A. brassicicola* colonies decreased after treatment with propamidine, indicating a decrease in melanin content. In addition, studies have

Fig. 4. Effect of propamidine on cell membrane permeability of mycelia of two wild-type *A. brassicicola* isolates (YL12 and RG5). Bars indicate the standard error of three experiments.

Fig. 5. Effect of propamidine on chitin content of two wild-type *A. brassicicola* isolates (YL12 and RG5). Asterisks indicate significant differences ($P < 0.05$) compared with the untreated control for each isolate.

Fig. 6. Effect of propamidine on the expression of genes associated with melanin biosynthesis in *A. brassicicola*. Asterisks indicate significant differences ($P < 0.05$) compared with the untreated control for each gene.

shown that the *Amr1*, *Scd1*, *Brn1*, and *Brn2* genes regulate melanin biosynthesis in *A. brassicicola*. The *Amr1* gene deletion mutant is melanin-deficient (Cho et al. 2012). Moreover, genes involved in melanin biosynthesis, including *Scd1*, *Brn1*, and *Brn2* genes, have been reported for *Bipolaris oryzae*, a rice pathogen closely related to *C. heterostrophus*, and for *C. lagenarium* (Kihara et al. 2004; Takano et al. 1997). Therefore, we quantified the expression levels of these four genes by qRT-PCR. After treatment with propamidine, the expression levels of these four genes were downregulated. These results indicated that propamidine inhibited melanin biosynthesis in our isolates of *A. brassicicola*, which may lead to the reduced tolerance of *A. brassicicola* to environmental stresses (such as UV radiation or extreme temperatures).

To our knowledge, this study is the first report the baseline sensitivity of *A. brassicicola* to propamidine and the significant potential value of this fungicide for controlling dark leaf spot on cabbage. Propamidine inhibited not only the mycelial growth of *A. brassicicola* but also melanin biosynthesis, which could reduce the biological fitness of *A. brassicicola* in field conditions. These results support the notion that propamidine may be a good alternative to commonly used fungicides for the management of dark leaf spot on cabbage. This information encourages further investigation of the mechanism of action of propamidine against other plant pathogens.

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