
Efficacies of Some Fungicides and Antagonists in Controlling Northern Corn Leaf Blight Disease

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Abstract Three fungicides (chlorothalonil, difenoconazole and mancozeb) at three concentrations: ½ lower than recommended rate, recommended rate and ½ higher than recommended rate, were tested on efficacy to inhibit growth of different *Exserohilum turcicum* isolates: MHP5, TN3, MJ4, JT4 and JT5, using the poisoned medium method. Results showed that all concentrations of the two contact fungicides, chlorothalonil and mancozeb, gave 100% growth inhibition to all five isolates. Whereas difenoconazole showed 100% growth inhibition in isolates MHP5, TN3 and MJ4 at all three concentrations but gave about 90% in JT4 and about 94-96% in JT5. A field trial on efficacy of the three fungicides at recommended rates was carried out in comparison with two antagonists: *Trichoderma harzianum* and *Serratia plymutica* in controlling northern corn leaf blight disease. Hibrix3 sweet corn plants (23 days old) were inoculated with *E. turcicum* (10^6 spore/ml) at either 3 d or 7 d after spraying with the fungicides and antagonists. Results revealed that both fungicides and antagonists gave high percentages of disease severity reduction at 10 d after inoculation of 89.58% (difenoconazole), 85.40% (*T. harzianum*), 79.84% (chlorothalonil), 77.78% (*S. plymutica*), and 75.69% (mancozeb), when sprayed 3 d before inoculation with the pathogen. The efficacies were increased in the corn plants that were sprayed at 7 d before inoculation, giving severity reductions of 92.35% (chlorothalonil), 91.66% (*T. harzianum*), 90.55% (*S. plymutica*), 89.85% (difenoconazole), and 86.11% (mancozeb). Disease control decreased at 20 d after inoculation but was still significant. Though the difenoconazole showed highest reduction percentages while mancozeb came second when applied at 3 or 7 d before pathogen inoculation, whereas disease reduction by both antagonists ranged from 62% to 70% which are lower than the chemical fungicides, anyhow there was no any statistical difference among the treatments.

Keywords: Northern Corn Leaf Blight, *Exserohilum turcicum*, Fungicides, Antagonists, Control

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Introduction

Corn plants have many diseases but northern corn leaf blight (NCLB) is most common and causes severe damage to this crop around the world (Harlapur *et al.*, 2007). The disease is caused by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs (syn. *Helminthosporium turcicum* Pass). In the humid areas wherever the corn is grown, the pathogen can infect the plant within 6 h after contact with the leaf surface and the symptoms are visible 3 d after inoculation (Lipps and Mills, 2002). The spindle-shaped lesions are light brown to dark brown with the width of about 1.5-15.0 cm parallel to the midrib. As many lesions enlarge and merge, entire leaves may be covered. The symptoms begin in the lower leaves and spread to the upper leaves. In the end, the whole plant dies with all the leaves become blighted. The pathogen's spores are produced and disseminated by wind and/or on contaminated seed. The best conditions for pathogen development are moderate temperature (22-30° C) and high relative humidity (90-100%). When outbreaks of the disease occurred in northern Thailand, yield reductions ranged from 40-80% (Sitthikul, 1996).

Schwartz and David (2005) studied the life cycle and seasonal history of *Helminthosporium* leaf blight. They stated that *Helminthosporium* leaf blight was a general term for diseases caused by several fungi formerly known as *Helminthosporium* spp. These diseases include northern corn leaf blight. Infection of susceptible varieties occurs when temperatures are moderate (18-28° C) to warm (20-32° C) and humidity is high. *Helminthosporium* leaf blight pathogens survive between corn crops as spores (conidia) and mycelium remaining in and on crop debris, but can also be transported long distances by wind. They suggested that fungicides for controlling of the leaf blight are chlorothalonil, EBDC-fungicides, propiconazole, azoxystrobin, anyhow for the high effective they should be combined with the varieties with general resistance.

Bowen and Pederson (1988) reported that propiconazole could inhibit *E. turcicum* mycelial growth but could not inhibit spore germination. Raid (1991) studied the efficacy of mancozeb, chlorothalonil and propiconazole for controlling rust and northern leaf blight in corn and found that all fungicides could reduce disease severity. In Thailand, Choosak and Thiwa (2003) reported that zineb, propineb and maneb were effective in controlling northern corn leaf blight.

Chlorothalonil was registered for NCLB control in the year 2007 but in 2010 propiconazole was approved and has more curative and systemic activities than chlorothalonil (Watson, 2007).

Harlapur *et al.* (2007) reported that *T. harzianum* was effective on growth inhibition *E. turcicum* (65.17%). They also reported that mancozeb was the most effective in growth inhibition. While carboxin powder at 0.1% and propiconazole at 0.1% were also effective disease-suppressive fungicides for sugarcane.

There are many methods for NCLB control but chemical fungicides are commonly used by growers. This is due to their rapid and high effectiveness. Besides, many kinds of fungicides are available to growers and easy to use compared to other procedures. However, selecting the most effective fungicide for manipulating the disease could help in cost reduction and saved time for the growers. Nowadays, most people realize the hazard of chemicals used in agriculture to human health. So, biological products for plant diseases and insect pests management have been produced and are available in the market. Much research on the use biological control has been carried out. *T. harzianum* is one of the most important antagonistic fungi; it can be used for controlling both air-borne and soil-borne diseases. *Serratia plymutica* is an antagonistic bacterium useful for controlling some fungal and bacterial plant diseases. The antagonists can destroy plant pathogens by producing enzymes, toxins and inhibitors.

The most successful NCLB control should be an integrated approach. So, the results of this study can give the growers, appropriate kinds of fungicide to spray for prevention the disease as Sommat (2000) suggested that spraying fungicides should be done for protection of the plants (contact fungicide) instead of letting the disease break out then spraying curatively. If the disease has been developed in the plant, systemic fungicide must be used; however, improper use of systemic fungicides could create fungicide resistant in pathogens.

Villa *et al.* (2006) reported that antagonist biocontroller of phytopathogenic fungi that there were many kinds of antagonistic fungi such as *Aspergillus* spp.: *A. versicolor*, *A. sacchari* and *A. nidulans*; and *T. harzianum* and *T. viride* including abiotic extracts from the fungi have been used to control many diseases. They also mentioned that these biocontrols were innocuous to man, environmental friendly and economically cheaper than chemicals products.

The bacterial antagonist *S. plymutica* can be isolated from soil around the roots of many plants e.g. grasses, corns, cabbages (Kurze, *et al.*, 2001) and was reported as a control agent for both soil- and air-borne pathogens.

Kurze *et al.* (2001) studied *S. plymutica* isolate HRO-C48 for controlling strawberry diseases e.g. *Verticillium dahliae* causing wilt disease and *Phytophthora cactorum* causing root rot in the planting plots by the root dipping technique. Results showed that the antagonist could reduce the wilt disease by

24.2% and root rot disease by 9.6%. It was found that the antagonist could survive in the rhizosphere for as long as 14 mo (David *et al.*, 2009)

Material and methods

Efficacy of three fungicides on growth inhibition of five isolates of *Exserohilum turcicum*

Preparation of the fungal pathogen

Five isolates of the fungus, *E. turcicum* e.g. MHP5, TN3, MJ4, JT4 and JT5 previously tested as virulent isolates, were used in this study. The fungal isolates were grown on potato dextrose agar (PDA) and active mycelia culture discs from the edge of the colonies of each isolate were used to inoculate poisoned PDA plates (Nene and Thapliyal, 1979).

Preparation of poisoned PDA plates

Three fungicides, two of which are contact fungicides – chlorothalonil (tetrachloroisophthalonitrile 75% WP) and mancozeb (manganese ethylene bis-dithiocarbamate with zinc salt 80% WP) and one systemic fungicide – difenoconazole (cis-trans-3-chloro-4 [4-methyl-2 (1H-1, 2, 4 -triazol-1ylmethyl)-1, 3-dioxolan-2-ylphenyl-4-chlorophenyl ether 25%] W/V EC.), were used in this study. Three rates of the fungicides were used: ½ of the producer recommended rate, recommended rate, and ½ higher than recommended rate. So the concentrations of the three fungicides used were as follows: chlorothalonil 375, 750 and 1,125 ppm; mancozeb 400, 800 and 1,200 ppm; difenoconazole 75, 150 and 225 ppm. The fungicides were dissolved in sterilized water before adding into melted PDA, using 5 ml of each fungicide at each concentration to mix with 70 ml of PDA in 100 ml flasks. So, the concentrations of each fungicide prepared were calculated to the concentrations required first and then made up to five times higher to meet the concentrations required in PDA. After shaking, the well mixed poisoned PDA was poured into five plates making five replications per treatment.

Testing effect of the fungicides on growth inhibition of five isolates of *E. turcicum*

One agar disc of the isolates MHP5, TN3, MJ4, JT4 and JT5 was placed in the middle of each poisoned PDA plate. The cultures were incubated at room temperature for 10 d.

Measurement of the fungal colonies

The diameter of the colonies was measured at 10 days after inoculation, when the control treatment sets were fully grown.

Calculation of the data

To calculate the data obtained from measuring the diameter of the fungal colonies of five isolates of *E. turcicum* in the control sets and in the treatments, the per cent inhibition of fungal growth was estimated by using the formula given by Vincent (1927):

$$\% \text{ growth inhibition} = \frac{C - T}{C} \times 100$$

Where C = colony diameter in control
T = colony diameter in treatment

A field trial of effectiveness of three fungicides and two antagonists for control of northern corn leaf blight

- *Preparation of planting plots and cultivation of corn plants*
- Land preparation was done by ploughing the soil and exposing it to the sunlight for one wk. planting plots were 2.25 x 3.52 m and separated by 1 m. Seeds of the Hibrix3 sweet corn variety were planted at a spacing of 25 x 75 cm, so there were 42 plots, and each plot had 36 plants.

Starkle G (dinotefuran) was put in the soil before sowing seeds for prevention of soil insects. The soil was also, nourished with 15-15-15 fertilizer (3 grams/planting hole). At 7 d of age, the plants were thinning to one plant/planting hole. When the corn plants reached the age of 20 d, they were fertilized for the second time with 46-0-0 fertilizer (2.5 grams/plant). At 23 d after germination, the plants were ready to be tested.

- *Preparation of inoculum*

The fungal pathogen, isolate MHP5, previously transferred from the stock culture, was grown on Vegetable-8 agar (V-8 agar) for 10 d. Spore suspensions were made to the concentration of 10⁶ spore/ml for inoculation.

- *Preparation of fungicides and antagonists*

Three fungicides at three recommended rates were prepared as described earlier (see 1.2). *T. harzianum* and *S. plymutica* from the stock culture of Plant Biotechnology Research Center, Chiang Mai University, were grown on potato dextrose agar (PDA) and nutrient broth (NB) respectively. The fungal spore suspension was adjusted to 10⁶ spore/ml and bacterial cell suspension was adjusted to 10⁶ cfu/ml.

Experimental design

The experiment was carried out in the field, using split plots arranged in a randomized complete block design (RCBD). There were three replications of seven treatments. Disease assessments were made on eight plants/treatment/replication. Details of the treatments are described below:

Treatment 1: Spraying the plants with mancozeb at 800 ppm before inoculation

Treatment 2: Spraying the plants with chlorothalonil at 750 ppm before inoculation

Treatment 3: Spraying the plants with difenoconazole at 150 ppm before inoculation

Treatment 4: Spraying the plants with cell suspension of *S. plymutica* (PBRC1), at concentration of 10^6 cfu/ml before inoculation

Treatment 5: Spraying the plants with spore suspension of *T. harzianum*, at concentration of 10^6 spore/ml before inoculation

Treatment 6: Spraying the plants with sterile water (Control 1)

Treatment 7: Spraying the plants with spore suspension of *E. turcicum*, at concentration of 10^6 spore/ml (Control 2)

Rating disease severity

Ratings of disease severity were made at 10 d and 20 d after inoculation. Percentages of infected leaf area and numbers of diseased leaves were used for scoring follow the NCLB scale and resistant level developed by Pataky (1992) as shown in Fig. 1.

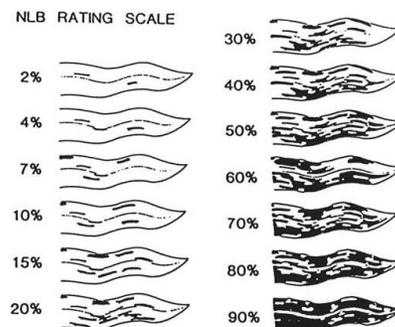


Figure 1. Disease rating pattern for evaluation of northern corn leaf blight disease caused by *Exserohilum turcicum* (after Pataky, 1992)

Resistant levels

Level 0 = No symptom

Level 1 = One leaf of the plant shows symptom (2-10% leaf area infection)

Level 2 = About 2-3 leaves of plant show symptom (10-15% leaf area infection)

Level 3 = All leaves of plant show symptom except apical leaf (30-40% leaf area

infection)

Level 4 = All leaves of plant show symptom (50% leaf area infection)

Level 5 = All leaves of plant show symptom or the whole plant dies. (70- 90% leaf area infection)

Results and discussion

Efficacy of three fungicides on growth inhibition of five isolates of Exserohilum turcicum

Results from cultivation of five isolates of *E. turcicum* on poisoned PDA (PDA mixed with each fungicide) showed that both contact fungicides, mancozeb and chlorothalonil, at three concentrations were highly effective on all the fungal isolates with 100% inhibition. While difenoconazole, a systemic fungicide, showed 100% inhibition to isolates MHP5, TN3 and MJ4 at all 3 concentrations, but on isolates JT4 the percentages of inhibition were 89-99% while inhibition percentages of JT5 were 94-96% (Table 1 and Fig. 2).

Table 1. In vitro effects of three fungicides incorporated in PDA on the growth of *Exserohilum turcicum*.

Fungicide	Concentration (ppm)	Percentage of growth inhibition ¹				
		MHP5	TN3	MJ4	JT4	JT5
Mancozeb	1,200	100±0.0 ^{a2}	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a
	800	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a
	400	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a
Chlorothalonil	1,125	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a
	750	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a
	375	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a
Difenoconazole	225	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	90.66±8.9 ^c	96.77±5.0 ^b
	150	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	89.77±9.6 ^c	96.22±5.3 ^b
	75	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	90.22±8.6 ^c	94.66±5.0 ^b
LSD ($p = 0.05$) main plot (fungal isolates)					0.9979	
LSD ($p = 0.05$) sub plot (concentrations of fungicides)					1.4112	
%CV					2.84	

¹ mean of 5 replications

² Means followed by the same letter in all columns are not significantly different by LSD ($p = 0.05$)

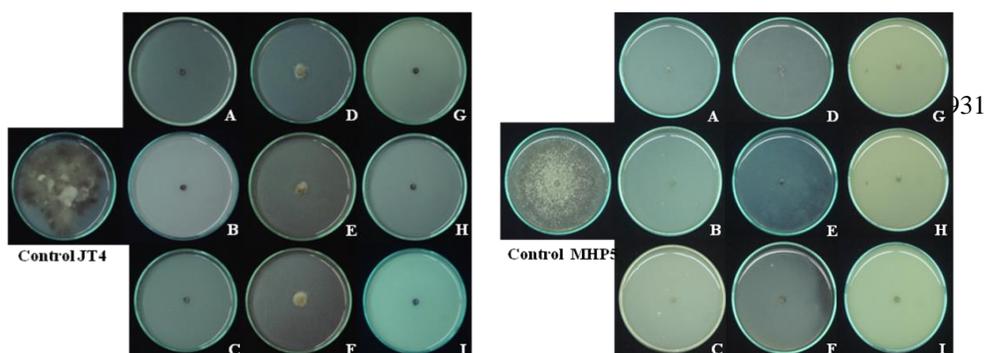


Figure 2. Growth inhibition of *E. turcicum*, isolates JT4 (left) and KHP5 (right) on PDA mixed with three fungicides at three concentrations compared with the control sets of the two isolates: Chlorothalonil at 375 ppm (A), 750 ppm (B), and 1,125 ppm (C); difenoconazole at 75 ppm (D), 150 ppm (E), and 225 ppm (F) and mancozeb at 400 ppm (G), 800 ppm (H) and 1,200 ppm (I), at 10 d after inoculation.

A field trial of the effectiveness of three fungicides and two antagonists for controlling northern corn leaf blight disease

Results from using three fungicides (mancozeb, chlorothalonil and difenoconazole previously tested on growth inhibition of the fungal isolates on poisoned PDA) in comparison with two antagonists (*S. plymuthica* and *T. harzianum*) for control of NCLB disease in Hibrix3 corn showed positive results as described below:

At 10 d after inoculation

Plants sprayed with difenoconazole, *T. harzianum*, chlorothalonil and *S. Plymutica* at 3 d before inoculation resulted in 89.58%, 85.4%, 79.84% and 77.78% disease severity reductions respectively which were not statistically different (LSD $p = 0.05$) from each other, while mancozeb had 75.69%, lowest disease reduction of all, however it was not statistically different from other treatments.

Plants sprayed with chlorothalonil and *T. harzianum* at 7 d before inoculation had the numerically highest percentages of disease severity reduction at 92.35% and 91.60%, respectively, while *S. plymutica*, difenoconazole and mancozeb had 90.55%, 89.85% and 86.11%, respectively; however, the disease reductions were not statistically different.

Table 2. Percentage of disease severity reduction in Hibrixs3 sweet corn hybrid inoculated with *E. turcicum*, resulting from spraying with three fungicides at three concentrations and two antagonists prior to inoculation, recorded at 10 d after inoculation.

Treatment	% reduction of disease severity ¹	
	3 d before inoculation	7 d before inoculation

mancozeb 800 ppm	75.69±27.68 ^{b2}	86.11±2.41 ^{ab}
chlorothalonil 750 ppm	79.84±14.64 ^{ab}	92.35±3.19 ^a
difenoconazole 150 ppm	89.58±3.60 ^{ab}	89.85±6.00 ^{ab}
<i>S.plymutica</i>10⁶cfu/ml	77.78±7.89 ^{ab}	90.55±5.29 ^{ab}
<i>T.harzianum</i>10⁶ spore/ml	85.4±3.61 ^{ab}	91.66±4.17 ^a
Control 1 (spraying water)	0.00±0.00 ^c	0.00±0.00 ^c
Control 2 (spraying inoculum)	0.00±0.00 ^c	0.00±0.00 ^c
LSD (<i>p</i> = 0.05) main plot (Period of spraying fungicides and antagonists)		5.76
LSD (<i>p</i> = 0.05) sub plot (Kind of fungicides and antagonists)		10.79
%CV		14.88
¹ mean of 3 replications		
² Means followed by the same letter within columns are not significantly different by LSD (<i>p</i> = 0.05)		

At 20 d after inoculation:

The plants treated only one time with the disease control agents at either 3 or 7 d before inoculation showed that the percentages of disease severity reduction by the two antagonists were lower than the fungicides, but they were not statistically different with the chemical treatments, especially the *T. harzianum* that gave lower inhibition efficacy than the others, anyhow after at 7 d, all of the treatments revealed no statistically difference in the inhibition efficacies. Though the difenoconazole showed the highest reduction percentages 83.33% (3 d) and 81.94% (7 d), whereas mancozeb ranked second with 79.17% (3 d) and 80.55% (7 d). The chlorothalonil revealed the lowest percentages among the three fungicides with 74.98% (3 d) and 76.39% (7 d), comparing with the reduction percentages of the biocontrol agents: *S. plymuthica* were 69.42% (3 d) and 65.28% (7 d) and *T. harzianum* were 62.45% (3 d) and 70.83% (7 d), as shown in Table 3, all the treatments did not show any statistical difference.

Table 3. Percentage of disease severity reduction in Hibrixx3 sweet corn hybrid inoculated with *E. turcicum*, resulting from spraying with three fungicides at three concentrations and two antagonists prior to inoculation, recorded at 20 d after inoculation.

Treatment	% reduction of disease severity ¹	
	3 d before inoculation	7 d before inoculation

mancozeb 800 ppm	79.17 ± 0.00 ^{abc2}	80.55 ± 6.36 ^{abc}
chlorothalonil 750 ppm	74.98 ± 12.53 ^{abcd}	76.39 ± 8.67 ^{abcd}
difenoconazole 150 ppm	83.33 ± 0.00 ^a	81.94 ± 12.03 ^{ab}
<i>S. plymutica</i> 10⁶ cfu/ml	69.42 ± 6.39 ^{cde}	65.28 ± 10.48 ^{de}
<i>T. harzianum</i> 10⁶ spore/ml	62.45 ± 0.00 ^e	70.83 ± 7.22 ^{bcd}
Control 1 (spraying water)	0.00 ± 0.00 ^f	0.00 ± 0.00 ^f
Control 2 (spraying inoculum)	0.00 ± 0.00 ^f	0.00 ± 0.00 ^f
LSD (<i>p</i> = 0.05) main plot (Period of spraying fungicides and antagonists)		4.20
LSD (<i>p</i> = 0.05) sub plot (Kind of fungicides and antagonists)		7.87
%CV		12.52
¹ mean of 3 replications		
² Means followed by the same letter within columns are not significantly different by LSD (<i>p</i> = 0.05)		

Conclusion

Two contact fungicides, chlorothalonil, mancozeb, and one systemic fungicide, difenoconazole, were tested for their efficacy to inhibit growth of five *E. turcicum* isolates using the poisoned PDA technique. The results showed that both contact fungicides at three tested concentrations (½ lower than producer recommended rate, recommended rate and ½ higher than recommended rate) gave 100% inhibition to isolate MHP5, TN3 and MJ4 and gave 89-90% inhibition to isolate JT4 and gave 94-96% reduction to JT5.

The three fungicides at recommended rates were also tested for their effectiveness in controlling NCLB disease caused by *E. turcicum* in a field trial in comparison with two antagonists, *S. plymutica* and *T. harzianum*. Both fungicides and the antagonists were sprayed on the corn plants at either 3 or 7 d before inoculation with *E. turcicum*. Results from the 10 d after inoculation indicated that the fungicides treatments gave very high percentages of disease severity reduction in the 7 d sub-treatment (86-92%) and high percentages in the 3 days sub-treatment (75-89%). At 20 d after inoculation, the highest percentages of disease reduction were obtained from the difenoconazole treatment in both 3 and 7 d sub-treatment (83.33-81.94%), whereas mancozeb was ranked second (79.17% and 80.55%), followed by chlorothalonil (74.98% and 76.39). The effectiveness of the two on disease severity reduction was equal to the three fungicides at 10 d after inoculation; *T. harzianum* impressively reduced blight by 91.66% when applied at 7 d before pathogen inoculation. Even though, both antagonists showed lower percentages of severity reduction than the three fungicides in the record at 20 d after inoculation: *S. plymutica* gave 69.42% (3 d) and 65.28% (7 d) while *T.*

harzianum gave 62.45% (3 d) and 70.83% (7 d) but they were not statistically different from the fungicide treatments.

It appears that management of NCLB by the fungicides and antagonists decreased between 10 and 20 dafter inoculation. This can be explained by the reduction of the toxic residues of the fungicides with time by rain or the sprinkler system and UV exposure. Besides, the spores of *E. turcicum* produced from the disease plants or other fields could reinfect the corn plants and develop symptoms continuously. The antagonists do not usually tolerate unfavorable conditions very long. Sunlight and drought can destroy the antagonists while the pathogen can persist under these conditions. Moreover, there also might be other microbes that are antagonistic to the applied antagonists (Chamswang *et al.*, 2003). The results of this work also indicated that spraying fungicides or antagonists prior to inoculation of pathogen could reduce disease severity as Sommat (2000) indicated. In order to control plant disease effectively, one must spray fungicides to prevent the plants from being attacked by the pathogen. Spraying before the symptoms appear can control the disease better than spraying after the pathogen has been well developed in the plant, and caused damage (Abebe and Singburaudom, 2006). Favorable conditions including low temperature and high humidity can promoting disease severity, enabling the pathogen to actively grow and produce more spores. Therefore, repeated spraying of antagonists under such disease-conducive conditions in order to lower the foliar infection of the pathogen is highly recommended.

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