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

Wathaneeyawech, S.¹, Sirithunya, P.² and Smitamana, P.^{3*}

¹Plant Biotechnology Program, Graduate School, Plant Biotechnology Research Centre, Chiang Mai University, Chiang Mai 50200, Thailand, ²Lampang Agricultural Research and Training Centre, Lampang, Thailand, ³Plant Biotechnology Research Centre, Chiang Mai University, Chiang Mai 50200, Thailand.

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Abstract Three fungicides – chlorothalonil, difenoconazole and mancozeb at 3 concentrations e.g. ½ lower than recommended rate, recommended rate and ½ higher than recommended rate were tested on efficacy to inhibit growth of isolates MHP5, TN3, MJ4, JT4 and JT5 of *E. turcicum*, using poisoned medium method. Results showed that the two contact fungicides, chlorothalonil and mancozeb at 3 concentrations gave 100% growth inhibition to all five isolates. Whereas difenoconazole showed 100% growth inhibition in isolates MHP5, TN3 and MJ4 at all 3 concentrations but gave about 90% in JT4 and about 94-96% in JT5. A field trial on efficacy of the three fungicides at recommended rate was carried out in comparison with two antagonists – *Trichoderma harzianum* (fungus) and *Serratia phymutica* (bacterium) in controlling northern corn leaf blight disease. The sweet corn (variety Hibrix3) plants (23 days old) were inoculated with *E. turcicum* (10^6 spores/ml) at 3 days and 7 days after spraying with the fungicides and antagonists. Results showed that both fungicides and antagonists gave high percentages of disease severity reduction at 10 days after inoculation ranges from 89.58% (difenoconazole), 85.40% (T. harzianum), 79.84% (chlorothalonil), 77.78% (S. phymutica) to 75.69% (mancozeb) in the set of 3 days. The efficacies were increased in the corn plants that were sprayed at 7 days before inoculation, giving severity reduction ranges from 92.35% (chlorothalonil), 91.66% (T. harzianum), 90.55% (S. phymutica), 89.85% (difenoconazole) to 86.11% (mancozeb). At 20 days after inoculation, percentages of the severity reduction were lower but still rather high. In both 3 days and 7 days, dificonazole showed highest percentages while mancozeb came second, both antagonists had about 62% to 70% reduction, lower than the chemical fungicides.

Keywords: *Colletotrichum gloeosporioides*, strawberry, anthracnose, filtered and nonfiltered bacterial extracts, biofungicides

Introduction

* Corresponding author: Smitamana, P.; E-mail: psporn@gmail.com

Corn plants have many diseases but northern corn leaf blight (NCLB) is most common and causes big damage to the growers around the world (Harlapur *et al.*, 2007). The disease is caused by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs (syn. *Helminthosporium turcicum* Pass). This pathogen can infect the plant within 5 hours after inoculation and the symptoms are shown in 3 days after inoculation. Observation of the symptoms of the diseased leaves indicated that there are many lesions on the heavily damaged leaves. The spindle-shaped lesions are light brown to dark brown with the width of about 1.5-15.0 centimeters parallel to the midrib. The symptoms began from the lower leaves and spreaded to the upper leaves. In the end, the whole plant died with all the leaves turned blight. The pathogen spores were produced and disseminated by wind and/or contaminated on seed. The best condition for pathogen development was at moderate temperature (22-30° C) with high relative humidity (90-100%). When the disease outbreak occurred in the region, the percentage reduction of yield have been ranged from 40-80 (Sitthikul, 1996).

Schwartz and David (2005) studied life cycle and seasonal history of *Helminthosporium* leaf blight. They stated that *Helminthosporium* leaf blight is 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 (64-81° F) to warm (68-90° F) and damp, humidity, weather prevails. *Helminthosporium* leaf blight pathogens survive between corn crops as spores (conidia) and mycelium in and on crop debris, but can also be transported long distances on wind currents. They suggested that fungicides for control of the leaf blight are chlorothalonil, EBDC, propiconazole, azoxystrobin, together with period of spraying.

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

Chlorothalonil was registered for NLB (NCLB) control in the year 2007 but in 2010 propiconazole was approved as more curative and systemic activities than chlorothalonil. (www.dpi.nsw.gov.au.primefacts)

Harlapur *et al.*, 2007 reported that *Trichoderma harzianum* was effective on growth inhibition at 65.17% they also reported that mancozeb showed most

effective on growth inhibition. While carboxin powder at 0.1% and propiconazole at 0.1% were also effective fungicides.

There are many methods for control of this disease but chemical fungicides are commonly used by growers. This is due to their effectiveness expressed more distinctly and more quickly. Besides, the fungicides are available in the shops, there are many kinds, and easy to use. However, selecting the most effective fungicide for control the disease could help reducing the cost and time for the growers. Nowadays, most people realize on hazard of chemicals used in agriculture to human health. So, biological products for control of plant diseases and insect pests have been produced and are available in the markets. Researches on biological control have been carried out simultaneously. *Trichoderma harzianum* is one of the most important antagonistic fungi; it can be used for controlling both air-borne and soil-borne diseases. *Serratia plymuthica* is an antagonistic bacterium useful for controlling some fungal and bacterial plant diseases. The antagonists can destroy the plant by producing enzyme, toxin and inhibitor.

Most successful control of NCLB should be 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 made for protection of the plants (contact fungicide) instead of letting the disease broke out then spraying. If the disease has been developed in the plant, systemic fungicide must be used but improper use of systemic fungicide could create resistance of the pathogen to the fungicide.

Villa *et al.* (2006) informed about antagonist biocontroller of phytopathogenic fungi that there are many kinds of antagonistic fungi such as *Aspergillus* spp.; *A. versicolor*, *A. sacchari* and *A. nidulans*; *Trichoderma harzianum*, *H. viride* including abiotic extracts from the fungi have been used for controlling many diseases. They also mentioned that these bicontrollers are innocuous to the man and they do not damage environment, they also economically cheaper than chemicals products.

The bacteria antagonist *S. plymuthica* can be isolated from soil around the roots of many plants e.g. grasses, corns, cabbages (Alstrom *et al.* 1987, Lucon and Melo, 2000). This bacterium was informed as a control agent for both soil-borne pathogen and air-borne pathogen.

Kurze *et al.* (2001) studied on *S. plymuthica* isolate HRO-C48 for control of strawberry diseases e.g. *Verticillium dahliae* causing wilt disease and *Phytophthora cactorum* causing root rot in the planting plots by root dipping technique. Results showed that the antagonist could reduce the wilt disease at 24.2% and root rot disease at 9.6%. Checking survival of the antagonist found that it could survive in the rhizosphere as long as 14 months (David *et al.* 2009).

Materials and methods

Experimental design

*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) until fill up the 9 cm Petridish. One culture disc of each isolate was used to inoculate on a poisoned PDA plate.

Preparation of poisoned PDA plates

Three fungicides, two of which are contact fungicide – chlorothalonil (tetrachloroisophthalonitrile 75% WP) and mancozeb (manganese ethylene bis-dithiocarbamate with zinc salt 80% WP) and the rest is 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. The rates of the fungicides used are varied to 3 concentrations; $\frac{1}{2}$ lower than recommended rate, recommended rate and $\frac{1}{2}$ higher than recommended rate. So the concentrations of the three fungicides used are 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 water before adding into sterilized melted PDA (warm PDA), using 5 ml of each fungicide at each concentration to mix with 70 ml of PDA in the 100 ml flask. So, the concentrations of each fungicide prepared were calculated to the concentrations required first and then made up to 5 times higher to meet the concentrations required in PDA. After shaking, the well mixed poisoned PDA was poured into 5 plates making 5 replications per treatment. Poison food technique (Nene and Thapliyal, 1979).

*Testing effect of the fungicides on growth inhibition of 5 isolates of *E. turcicum**

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

Measurement of the fungal colonies

The diameter of the colonies were 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

The soil was ploughed and exposed to the sunlight for one week. Then the planting plots were made to the size of 2.25 x 3.52 meters with one meter apart. The plots were prepared to grow the sweet corn plant (Hibrix3) at spacing of 25 cm between plants and 75 cm between rows. There were 42 plots, each plot had 36 plants.

Prevention of soil insect, starkle G (dinotefuran) was put in the soil before sowing seeds. The soil was also, nourished with 15-15-15 fertilizer (3 grams/planting hole). At 7 days of age the plants were thinning to have one plant/planting hole. When the corn plants reached the age of 20 days, they were fertilized with 46-0-0 fertilizer for the second time (3 grams/plant). At 23 days 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 about 10 days. Spore suspension was made to the concentration of 10^6 spores/ml for inoculation.

Preparation of fungicides and antagonists

Three fungicides e.g. chlorothalonil, mancozeb and difenoconazole at 3 recommended rated were prepared as described earlier (see 1.2). Two antagonists – *Trichoderma harzianum* (antagonistic fungus) and *Serratia phymutica* (antagonistic bacterium) from the stock culture of Research Center and Biotechnology of Plants, Chiang Mai University, were grown on potato dextrose agar (PDA) and nutrient broth (NB) respectively. The fungal spore suspension was adjusted to 10^6 spores/ml and bacterial cell suspension was adjusted to 10^6 cfu/ml

Experimental design

The experiment was carried out in the field, using split plot in randomized complete block design (RCBD). There were 3 replications of 7 treatments. Scorings were made on 8 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 difenocpnazole at 150 ppm before inoculation

Treatment 4: Spraying the plants with cell suspension of *S. phymutica* (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 spores/ml. before inoculation

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

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

Rating disease severity

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

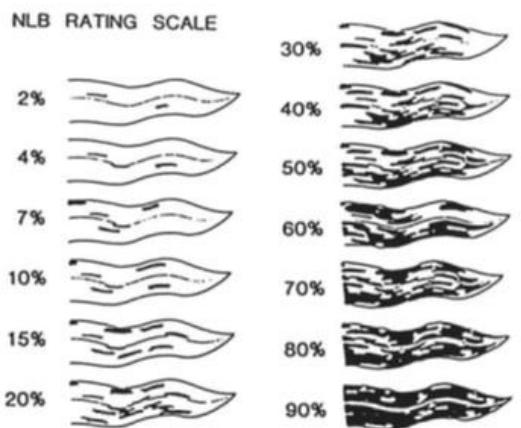


Fig. 1.Disease rating pattern for evaluation of northern corn leaf blight disease caused by *Exserohilum turcicum* (Pataky, 1992)

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 discussions

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

Results from cultivation of 5 isolates of *E. turcicum* on poisoned PDA (PDA mixed with each fungicide) showed that both contact fungicides – mancozeb and chlorothalonil at 3 concentrations are highly effective on all the fungal isolates with 100% inhibition. While dificonazole, 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 at 89-99% while inhibition percentages of JT5 were at 94-96% (Table 1 and Fig. 2).

Table 1. Percentage of growth inhibition of five isolates of *Exserohilum turcicum* on PDA mixed with each of three fungicides at three concentrations

Fungicide	Concentration (ppm)	Percentage of growth inhibition ¹				
		MHP5	TN3	MJ4	JT4	JT5
Mancozeb	1,200	100±0.0 ^a ²	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 ($p = 0.05$)

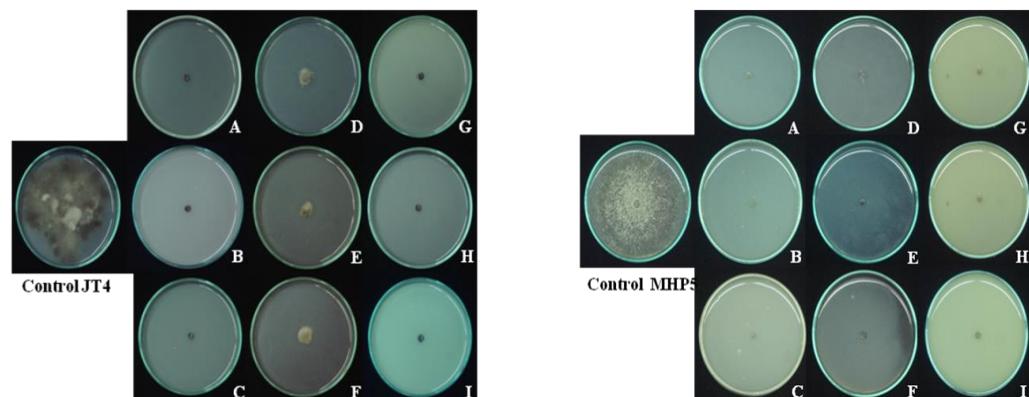


Fig. 2. Growth inhibition of *Exserohilum turcicum*, isolates MHP5 (left) and JT4 (right) on PDA mixed with three fungicides at 3 concentrations compared with the control sets of the two isolates: Chlorothalonil at 375 ppm (A), 750 ppm (B), and 1,125 ppm (C); difenoconazole at 75 ppm (D), 150 ppm (E), and 225 ppm (F) and mancozeb at 400 ppm (G), 800 ppm (H) and 1,200 ppm (I), at 10 days after inoculation.

A field trial of effectiveness of three fungicides and two antagonists for control of 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 sweet corn (Hibrix3) showed positive results as described below:

At 10 days after inoculation

The plants sprayed with difenoconazole, *T. harzianum*, chlorothalonil and *S. phymutica* at 3 days before inoculation had 89.58%, 85.4%, 79.84% and 77.78% reduction of disease severity respectively which are not statistically different (LSD $p = 0.05$) from each other. While mancozeb had 75.69%, lowest of all, and is statistically different from other treatments.

The plants sprayed with chlorothalonil and *T. harzianum* at 7 days before inoculation had highest percentages of reducing disease severity at 92.35% and 91.60% respectively (not statistically different between the two treatments). While *S. phymutica*, difenoconazole and mancozeb had 90.55%, 89.85% and 86.11% respectively (not statistically different among them).

When the percentages of reducing disease severity of the treatments at 3 days before inoculating the fungal pathogen were compared with the treatments at 7 days, the latter had higher percentages of reduction as shown in Table 2.

Table 2. Percentage reduction of disease severity in sweet corn inoculated With *E. turcicum*, resulted from spraying with 3 fungicides at three concentrations and 2 antagonists prior to inoculation, recording at 10 days after inoculation

Treatments	% reduction of disease severity ¹	
	3 days before inoculation	7 days 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>Serratia plymuthica</i>	77.78 ±7.89 ^{ab}	90.55 ±5.29 ^{ab}
<i>Trichoderma harzianum</i>	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 ($p = 0.05$) main plot (Period of spraying fungicides and antagonists(5.769
LSD ($p = 0.05$) sub plot) Kind of fungicides and antagonists(10.793
%CV		14.88

¹ mean of 3 replications

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

At 20 days after inoculation

The plants treated with the disease control agents at 3 and 7 days before inoculation; results showed that percentages of severity reduction of the two antagonists are lower than fungicides. The reduction percentages of *S. plymuthica* are 69.42% (3 days) and 65.28% (7 days) while percentages of *T. harzianum* are 62.45% (3 days) and 70.83% (7 days). Difenoconazole gave highest reduction percentages at 83.33% (3 days) and 81.94% (7 days). Whereas mancozeb came second with 79.17% (3 days) and 80.55% (7 days). Chlorothalonil gave lowest percentages among the three fungicides with 74.98% (3 days) and 76.39% (7 days) (Table 3).

Table 3. Percentage reduction of disease severity in sweet corn inoculated with *E. turicum*, resulted from spraying with 3 fungicides at three concentrations and 2 antagonists prior to inoculation, recording at 20 days after inoculation

Treatments	% reduction of disease severity ¹					
	3 days before inoculation		7 days before inoculation		7 days before inoculation	
mancozeb 800 ppm	79.17 \pm 0.00 ^{abc2}		80.55 \pm 6.36 ^{abc}			
chlorothalonil 750 ppm	74.98 \pm 12.53 ^{abcd}		76.39 \pm 8.67 ^{abcd}			
difenoconazole 150 ppm	83.33 \pm 0.00 ^a		81.94 \pm 12.03 ^{ab}			
<i>Serratia plymuthica</i>	69.42 \pm 6.39 ^{cde}		65.28 \pm 10.48 ^{de}			
<i>Trichoderma harzianum</i>	62.45 \pm 0.00 ^e		70.83 \pm 7.22 ^{bcd}			
Control 1 (spraying water)	0.00 \pm 0.00 ^f		0.00 \pm 0.00 ^f			
Control 2 (spraying inoculum)	0.00 \pm 0.00 ^f		0.00 \pm 0.00 ^f			
LSD ($p = 0.05$) main plot (Period of spraying fungicides and antagonists)	4.207					
LSD ($p = 0.05$) sub plot (Kind of fungicides and antagonists)		7.870				
%CV			12.52			

¹ mean of 3 replications

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

Conclusion

Two contract fungicides – chlorothalonil, mancozeb and one systemic fungicide – dificonazole were tested on their efficacy to inhibit growth of 5 isolates of *E. turicum* on poisoned PDA. Results showed that both contract fungicides at 3 concentrations ($\frac{1}{2}$ lower than recommended rate, recommended rate and $\frac{1}{2}$ 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.

For the field trial, the three fungicides at recommended rate were tested on their effectiveness in controlling NCLB disease caused by *E. turcicum* in comparison with two antagonists, *Serratia plymuthica* (bacterium) and *Trichoderma harzianum* (fungus). Both fungicides and the antagonists were sprayed to the corn plants at 3 days and 7 days before inoculation with *E. turcicum*. Results from recording at 10 days after inoculation indicated that the treatments, on using fungicides gave very high percentages of disease severity reduction in the 7 days sub-treatment (86-92%) and high percentages in the 3 days sub-treatment (75-89%). At 20 days after inoculation, the data recorded showed highest percentages of dificonazole in the 3 days and 7 days sub-treatment for reduction of disease severity (83.33-81.94%). While mancozeb came second (79.17% and 80.55%), followed by chlorothalonil (74.98% and 76.39). For the antagonists, their effectiveness on disease severity reduction was even higher than the fungicide difenoconazole and mancozeb in the record made at 10 days after inoculation (*Trichoderma harzianum* at 91.66%) in the 7 days sub-treatment. However, both antagonists showed lower percentages of severity reduction than the three fungicides in the record at 20 days after inoculation: *Serratia plymuthica* gave 69.42% (3 days) and 65.28 (7 days) while *Trichoderma harzianum* gave 62.45% (3 days) and 70.83 (7 days).

It could be seen that percentages of disease severity reduction recorded at 20 days after inoculation were lower compared to the record at 10 days. This can be explained that toxic residues of the fungicide are reducing steadily with time. Whereas, fungal spores of the pathogen from the field could land on the corn plants and develop symptoms at all times. For the antagonists, they do not usually stand for unfavorable condition very long. Sunlight, drought can destroy the antagonists while the pathogen can persist in that condition. Moreover, there might be other microbes that are antagonistic to the antagonists as well (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) informed that to control plant disease effectively; one must spray fungicide to prevent the plants from being attacked by the pathogen. Spraying before the symptoms appear could control the disease better than spraying after the pathogen had been well developed in the plant and the symptoms were already shown (Abebe and Singburaudom, 2006). Favorable conditions including low temperature and high humidity can promote disease severity, making the pathogen grows well and reproduces more spores. As the disease spreads more quickly, the chemical fungicides and the antagonists are less effective when the time passed by Chana, 1988.

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