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Effect of different active ingredients of fungicides on *Alternaria* spp. growth *in vitro*

E. Survilienė and E. Dambrauskienė

Lithuanian Institute of Horticulture, LT-54333 Babtai, Kaunas distr., Lithuania;
e-mail: e.surviliene@lsdi.lt

Abstract. The impact of different active ingredients of fungicides on the development of *Alternaria* species *in vitro* was estimated by using Amistar 250 SC (azoxystrobin), Signum 334 WG (boscalid, pyraclostrobin), Zato 50 WG (trifloxystrobin) and Folicur 250 EW (tebuconazol). The growth colonies of micromycetes *Alternaria alternata* (isolated from *Thymus vulgaris*, *Levisticum officinale*), *Alternaria alternata, Alternaria brassicae* (isolated from *Brassica oleracea* convar. *capitata* var. *alba*) and *Alternaria dauci* (isolated from *Daucus carota*) were tested on the potato dextrose agar medium with additions of fungicides. All tested fungicides showed sufficient inhibitory activity on the growth *Alternaria* spp. colonies, , which decreased on average from 94-25% over 21 days in comparison with the control.

Key words: *Alternaria alternata, Alternaria brassicae, Alternaria dauci,* growth inhibition, fungicides

INTRODUCTION

Fungi of *Alternaria* Nees genus are widely spread as pathogens on field vegetables, ornamental and orchard plants and cause substantial yield losses in a broad range of host crop species (Stranberg, 1992; Farrar et al., 2004). Their polyphagous nature and ability to produce mycotoxins and other toxic metabolites means that they are potentially dangerous food spoilage agents (Repečkienė et al., 2005; Solfrizzo et al., 2005). Alternaria diseases decrease nutritive value of vegetables, their storability and resistance to rot (Azevedo et al., 2000; Šidlauskienė & Survilienė, 2002). Isolations from diseased plants showed that *Alternaria radicina* and *Alternaria dauci* attack carrots at all growing stages (Coles & Wicks, 2003; Farrar et al., 2004; Survilienė et al., 2006). Dark leaf spot (*Alternaria brassicae* and *Alternaria brassicicola*) was detected as the most harmful cabbage disease in commercial crops in Lithuania, damaging plants of different species and cultivars by 3.12–69.79% (Surviliene et al, 2004). *Alternaria* genus was the most common among fungi species isolated from sterilized and non-sterilized seeds of officinal and spice plants as well as vegetables (Stakvilevičienė & Grigaliūnaitė, 2003). Five species ascribed to the genus *Alternaria* were isolated from desiccated lovage and thyme herbs and *A. alternata* dominant (Repečkienė et al., 2005). Alternaria diseases are controlled through integrated use of clean seeds, sanitation, crop rotation, cultivar selection, and fungicide applications (Soteros, 1979; Babadoost et al.; 1993; Surviliene et al., 2005, 2006). Under high disease pressure, no single control measure is sufficient to manage the disease adequately. Multiple applications of fungicides are required to achieve economic yield and acceptable quality in infected crops, but there is always a possibility of finding pathogens resistance to pesticides.

The aim of the investigation was to evaluate the effect of fungicides with different active ingredients on the growth of *Alternaria* spp. colonies *in vitro.*

MATERIALS AND METHODS

The investigation was carried out at the Lithuanian Institute of Horticulture in 2005. The impact of different active ingredients of fungicides was estimated on the development of the following *Alternaria* species *in vitro*: *A. dauci* (Kühn) Groves et Skolko (7 strains *A. dauci*–Cr isolated from carrot *Daucus carota* L.); *A. brassicae* (Berk.) Sacc. (6 strains *A. brassicae*–Cb isolated from cabbage *Brassica oleracea* L**.** convar. *capitata* (L.) Alef. var. *alba* DC) and *A. alternata* (Fr.) Keissl. (3 strains *A. alternata*–Th isolated from thyme *Thymus vulgaris* L., 2 strains *A. alternata*–Lv isolated from lovage *Levisticum officinale* W.D.J. Koch and 2 strains *A. alternata*–Cb isolated from cabbage). *Alternaria* spp. was collected from harvestable vegetative parts of vegetables at the stage of development and from desiccated spice herbs. Isolates were directly transferred to pure culture. Pure micromycetes strains were tested on the potato dextrose agar (pH 4.5-5.0) with the addition of 2.5 g^{-1} azoxystrobin (Amistar 250 SC), 3.34 g^{-1} (2.67 g^{-1} boscalid and 0.67 g^{-1} pyraclostrobin) (Signum 334 WG), 5 g^{-1} trifloxystrobin (Zato 50 WG) and 2.5 g^{-1} tebuconazol (Folicur 250 EW). Petri dishes with cultures were incubated in a thermostat at 26 ± 2°C temperature for 21 days. Assessments of the inhibitory activity were carried out after 7, 14 and 21 days. Each test was replicated four times. The data were subjected to ANOVA, and the means were separated by the least significant difference (LSD) test.

RESULTS AND DISCUSSION

The growth of colonies in diameter of tested *Alternaria* spp. strains on PDA medium with additions of fungicides was statistically different in comparison to control (Table 1). It should be noted that the most intensive inhibition of the growth of colonies of *A. dauci*–Cr, *A. brassicae*–Cb and *A. alternata*–Cb strains showed boscalid, pyraclostrobin (Signum) and tebuconazol (Folicur). Signum was also sufficiently effective to inhibit the growth colonies of *A. alternata*–Lv and *A. alternata*–Th strains. The most powerful inhibitory activity of fungicides was detected during the first 7 days of incubation. The growth of strain *A. dauci*–Cr was inhibited by exploratory active ingredients, on average up to 92.12%, *A. brassicae*–Cb - 85.05%, *A. alternata*–Cb – 88.64%, *A. alternate*–Lv - 95.0% and *A. alternata*–Th - 95.84% in comparison to control (Table 2).

After 14 days of incubation Folicur was distinguished for its inhibitory activity on *A. dauci*–Cr up to 70.0%, on *A. brassicae*–Cb - 67.34%, on *A. alternata*–Cb - 73.91% and statistically varied from other fungicides. Active ingredients boscalid, pyraclostrobin (Signum) and tebuconazol (Folicur) were distinguished for their inhibitory activity on *Alternaria* spp. colonies growth throughout and persisted till 71% and 62% after 21 days accordingly. In 7 days, Trifloxystrobin (Zato) controlled *Alternaria* spp. strains growth most efficiently (inhibitory activity an average of 92.52%).

Table 1. Impact of fungicides on *Alternaria* species colonies growth *in vitro.*

Strain of *Alternaria* spp.	Incubation time in days	Diameter of colony, mm				
		Control	Amistar 2.5 g^{-1}	Zato 5 g^{-1}	Signum 3.34 g^{-1}	Folicur 2.5 g^{-1}
A. dauci–Cr	7	6.03 a	0.4 c	0.37 c	0.63 b	0.5 b
	14	8.9 a	5.17 b	5.53 b	3.67 c	2.67 c
	21	9.0 a	7.8 b	7.7 b	3.97 c	2.93 c
A. brassicae–Cb	7	5.3 a	0.67 b	0.43 c	1.37 b	0.7 b
	14	8.97 a	5.2 b	5.03 b	4.17 b	2.93 c
	21	9.0 a	6.17 b	6.07 b	4.63 c	3.43 c
A. alternata–Cb	7	3.3 a	0.43 b	0.27 c	0.5 b	0.3 c
	14	8.93 a	5.5 b	5.43 b	3.07 c	2.33 c
	21	9.0 a	6.77 b	6.43 b	3.87 c	3.9 c
A. alternata–Lv	7	3.1 a	0.1 b	-	0.2 b	-
	14	9.0 a	4.5 b	-	1.2 c	-
	21	9.0 a	6.5 b	-	1.8 c	-
A. alternata–Th	7	6.0 a	0.2 b	-	0.3 b	-
	14	9.0 a	4.5 b	-	1.6 c	-
	21	9.0 a	6.7 b	-	1.8 c	-

Note: means followed by the same letter in rows were not significantly different according to LSD means separation test (P = 0.05).

Table 2. Inhibitory activity of fungicides on *Alternaria* species colonies growth *in vitro.*

Strain of *Alternaria* spp.	Incubation time in days	Inhibitory activity, %			
		Amistar 2.5 g^{-1}	Zato 5 g^{-1}	Signum 3.34 g^{-1}	Folicur 2.5 g^{-1}
A. dauci–Cr	7	93.37 a	93.86 a	89.55 a	91.71 a
	14	41.91 c	37.87 c	58.76 b	70.00 a
	21	13.33 b	14.44 b	55.89 a	67.44 a
A. brassicae–Cb	7	87.36 a	91.89 a	74.15 b	86.79 a
	14	42.03 c	43.92 c	53.51 b	67.34 a
	21	31.44 c	32.56 c	48.56 b	61.89 a
A. alternata–Cb	7	86.97 a	91.82 a	84.85 a	90.91 a
	14	38.41 c	39.19 c	65.62 b	73.91 a
	21	24.78 b	28.56 b	57.00 a	56.67 a
A. alternata–Lv	7	96.67 a	-	93.33 a	-
	14	50.00 b	-	86.67 a	-
	21	27.78 b	-	80.00 a	-
A. alternata–Th	7	96.67 a	-	95.00 a	-
	14	50.00 b	-	82.22 a	-
	21	25.56 b	-	80.00 a	-

After 21 days its activity was observed on *A. brassicae* – Cb (inhibitory activity an average 32.56%), but the influence on the growth of other strains was low. Analogical action on mycelium growth of *Alternaria* spp. strains was observed in the case with azoxystrobin (Amistar). The progress (?) of the effect of pesticides depends on resistance of pathogens. Strobilurin fungicides have become a very valuable tool for managing diseases. In the past few years, azoxystrobin and pyraclostrobin have shown excellent control of Alternaria diseases in vegetable crops (Šidlauskienė et al., 2003; Farrar et al., 2004; Survilienė et al., 2006). However, it is noted that there is a possibility for some fungi in the population of *Alternaria* species to show fungicide resistance (Farrar et al., 2004). In our tests, strains *A. dauci*–Cr showed resistance to azoxystrobin (Amistar) and trifloxystrobin (Zato) after 21 days of incubation, inhibitory activity was 13.33 and 14.44%.

CONCLUSIONS

1. All tested fungicides showed sufficient inhibitory activity on the growth of *Alternaria* spp. Colonies, which decreased on average from 94–25% over 21 days in comparison to control.
2. It should be noted that active ingredients boscalid, pyraclostrobin (Signum 334 WG) and tebuconazol (Folicur 250 EW) were distinguished for their inhibitory activity on the growth of *Alternaria* spp. colonies throughout and persisted until 71% and 62% after 21 days accordingly.

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