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In vitro efficacy of fungicides against *Alternaria alternata* (Fr.) Keissler causing Alternaria blight of fennel

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Abstract

Fennel (*Foeniculum vulgare* Mill.) is an important spice crop known to infect by the fungal pathogen [*Alternaria alternata* (Fr.) Keissler] which is a limiting factor for low productivity of the fennel seeds. Looking to the regular occurrence of disease in moderate to severe form in Saurashtra region and the seriousness of the disease, the present study was carried out to evaluate efficacy of different fungicides against the test pathogen *in vitro*. The efficacy of different non-systemic fungicides at 1000, 1500 and 2000 ppm; systemic fungicides at 100, 250 and 500 ppm and ready-mixed fungicides at 500, 750 and 1000 ppm concentrations were tried against *A. alternata* using Poisoned Food Technique. Among non-systemic fungicides, copper hydroxide 53.8 DF at 2000 ppm concentration gave 93.74 percent mycelial growth inhibition. But, it was remained statistically at par with copper oxychloride 50 WP at same concentration with 93.19 percent mycelial growth inhibition. In contrast, chlorothalonil 75 WP noted 47.63 percent mycelial growth inhibition at 1000 ppm concentration. Whereas, among systemic fungicides, propiconazole 25 EC and tebuconazole 25.9 EC each at 500 ppm concentration remained equally effective and significantly superior over rest of the treatments with mycelial growth inhibition of 94.11 percent. On the other hand, thiophanate methyl 70 WP showed least effective at 100 ppm concentration (27.97%). Tebuconazole 50 + trifloxystrobin 25 WG at 1000 ppm concentration proved significantly superior over rest of the treatments with 93.75 percent mycelial growth inhibition. But, it was remained statistically at par with hexaconazole 4 + zineb 68 WP at 1000 and 750 ppm concentration with 93.56 and 93.00 percent mycelial growth inhibition, respectively. Whereas, carbendazim 12 + mancozeb 63 WP at 500 ppm concentration remained less effective as compared to other treatments and had minimum mycelial growth inhibition of 73.57 percent. But, it was remained statistically at par with kresoxim methyl 15 + chlorothalonil 56 WG at the same concentration with 74.50 percent mycelial growth inhibition.

Keywords: Fennel, Alternaria blight, *Alternaria alternata*, fungicides, mycelial growth inhibition

Introduction

Fennel is one of the important major seed spice crop, botanically named as *Foeniculum vulgare* Mill. belongs to the family Umbelliferae (Apiaceae) having chromosome number of $2n = 22$. The generic name derives from the Latin "Foenum", which means 'hay', referring to the foliar structure. It is a well-known aromatic medicinal plant used in traditional medicine and also as spice and substrate for different industrial purpose (Telci *et al.*, 2009) [12]. Vernacularly it is called Saunf or Badi Saunf.

In India, its seeds are used for mastication and chewing alone or with betel leaves. Fennel is believed to be native of Southern Europe and Mediterranean region. It is widely cultivated throughout the temperate and subtropical region of the world (Kusuma *et al.*, 2019) [9].

Fennel thrives well on drained loamy soil, which is rich in plant nutrients and lime. However, heavy soils are more desirable than light soils. Fennel is a cool season crop mainly grown during winter season in north India and does not thrive in south India except at higher elevation. A dry and cold weather favours higher seed production (Anon., 2020) [1].

Fennel is attacked by a number of diseases viz., Ramularia blight (*Ramularia foeniculi* Sibilla), powdery mildew (*Leveillula taurica* (Lév.) G. Arnaud, seedling damping off (*Alternaria petroselini* (Neerg.) E. G. Simmons), root rot (*Fusarium solani* (Mart.) Sacc.), Alternaria blight (*Alternaria alternata* (Fr.) Keissler and *Alternaria petroselini* (Neerg.) E.G.

Simmons). Among them, *Alternaria* blight of fennel caused by *A. alternata* is a serious bottleneck in augmenting fennel production in Gujarat (Chaudhari and Patel, 1987) [3]. The disease manifests itself on all above ground plant parts in the form of angular, black depressed lesions on the basal leaves with small dark spots that soon developed into necrotic areas on the fennel stalks (Infantino *et al.*, 2009) [5]. Looking to the regular occurrence of disease in moderate to severe form in Saurashtra region and the seriousness of the disease, it is necessary to study basic aspects of this disease particularly the efficacy of different fungicides in managing the disease under laboratory conditions intern to generate scientific information for its management under field condition and to minimize the crop losses caused by the disease.

Materials and Methods

The experiment was conducted in laboratory condition at the Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh during the year 2024-25. Seven different non-systemic, systemic and ready-mixd fungicides each with three different concentrations were tested against *A. alternata* using Poisoned Food Technique of Bagchi and Das (1968) [2] using Potato Dextrose Agar as a germinating medium. The experiment was laid out in Completely Randomized Design (factorial concept) with three repetitions.

A. Collection of samples

Samples were collected from naturally infected fennel plant with *Alternaria* blight disease from Research Farm of Department of Plant Pathology, JAU, Junagadh.

B. Isolation and purification

Isolation of fungus was made by standard tissue isolation method on Potato Dextrose Agar (PDA) medium in Petri plate (90 mm diameter) and incubated at 28±1 °C. The resulting fungal culture was purified by using the single spore isolation technique.

C. Fungicides used

Seven non-systemic fungicides *viz.*, chlorothalonil 75 WP, copper hydroxide 53.8 DF, copper oxychloride 50 WP, mancozeb 75 WP, metiram 75 WG, propineb 70 WP and zineb 75 WP with three concentrations (1000, 1500 and 2000 ppm); Seven systemic fungicides *viz.*, azoxystrobin 23 SC, carbendazim 50 WP, difenoconazole 25 EC, tebuconazole 25.9 EC, kresoxim-methyl 44.3 SC, propiconazole 25 EC and thiophanate methyl 70 WP with three concentrations (100, 250 and 500 ppm) and seven ready-mixed fungicides *viz.*, azoxystrobin 18.2 + difenoconazole 11.4 SC, metiram 55 + pyraclostrobin 5 WG, carbendazim 25 + mancozeb 50 WS, fluxapyroxad 25 + pyraclostrobin 25 SC, hexaconazole 4 + zineb 68 WP, kresoxim-methyl 15 + chlorothalonil 56 WG and tebuconazole 50 + trifloxystrobin 25 WG with three concentrations (500, 750 and 1000 ppm) were used.

D. Poison Food Technique

Appropriate quantity of each fungicide required was incorporated into autoclaved 100 ml PDA medium in 250 ml conical flask before solidification using digital balance

and micropipette for solid and liquid formulation of fungicide, respectively. After thoroughly shaking on the vertex, the medium was poured into sterilized Petri plates (90 mm dia.) in equal quantity (20 ml per Petri plate) to form a uniform layer. The plates were then allowed to solidify. After solidification, an actively growing mycelial bit of 4 mm diameter cut with the help of sterilized cork-borer were transferred under aseptic conditions in an inverted position in the center of the plate to make a direct contact with solidified PDA medium. Then Petri plates were incubated at 28±1 °C till the control plate attains full growth and observations were recorded on linear mycelial growth in treated and control plates. Inoculated Petri plates containing PDA medium without fungicides were served as control (Grover and Moore, 1962) [4].

E. Observations noted

The percent growth inhibition of the fungus in each of the treatments were calculated by using the following formula (Vincent, 1947) [13].

$$I = \frac{C - T}{C} \times 100$$

Where,

I = percent inhibition of mycelial growth

C = Average radial growth of fungus in control plate (mm)

T = Average radial growth of fungus in treated plate (mm)

Results and Discussion

The observations on mycelial growth inhibition were recorded after nine days of inoculation.

A. Efficacy of non-systemic fungicides

The data presented in Table 1, Figure 1 and Plate I revealed that all non-systemic fungicides were capable of reducing the mycelial growth of test pathogen as compared to control. Among different non-systemic fungicides tried *in vitro*, the maximum mean mycelial growth inhibition was observed in copper hydroxide 53.8 DF (92.02%). But, it was remained statistically at par with copper oxychloride 50 WP (90.98%). Chlorothalonil 75 WP remained the least effective treatment with mean mycelial growth inhibition of 56.16 percent. While, looking to the interaction effect between the non-systemic fungicides and various concentrations, copper hydroxide 53.8 DF at 2000 ppm concentration found significantly superior over rest of the treatments with 93.74 percent mycelial growth inhibition. But, it was remained statistically at par with copper oxychloride 50 WP at same concentration with 93.19 percent mycelial growth inhibition. Chlorothalonil 75 WP remained the least effective at all three concentrations tried with minimum mycelial growth inhibition of 62.27, 58.57 and 47.63 percent.

The results obtained were in accordance with the findings of Shekhada *et al.* (2023) [11] and Rani *et al.* (2018) [10]. They found copper oxychloride 50 WP and mancozeb 75 WP as the most effective treatment against *A. alternata*. Whereas, chlorothalonil 75 WP showed the least effective as per finding of Kantwa *et al.* (2014) [7] and Rani *et al.* (2018) [10] while working with leaf blight of groundnut and pigeonpea, respectively.

Table 1: Efficacy of non-systemic fungicides against *A. alternata* *in vitro*

Non-systemic fungicides	Mycelial growth inhibition (%)			Mean mycelial growth inhibition (%)
	1000 ppm	1500 ppm	2000 ppm	
Chlorothalonil 75 WP	43.64 (47.63)	49.93 (58.57)	52.11 (62.27)	48.56 (56.16)
Copper hydroxide 53.8 DF	71.97 (90.42)	73.47 (91.90)	75.51 (93.74)	73.65 (92.02)
Copper oxychloride 50 WP	70.24 (88.57)	72.71 (91.16)	74.87 (93.19)	72.61 (90.98)
Mancozeb 75 WP	59.55 (74.31)	62.05 (78.03)	66.66 (84.31)	62.75 (78.88)
Metiram 70 WG	57.64 (71.35)	62.55 (78.76)	64.27 (81.16)	61.49 (77.09)
Propineb 70 WP	57.88 (71.73)	59.42 (74.12)	61.66 (77.47)	59.65 (74.44)
Zineb 75 WP	59.31 (73.95)	60.41 (75.62)	61.55 (77.30)	60.42 (75.62)
Mean	60.03 (73.99)	62.93 (78.31)	65.23 (81.35)	-
	Fungicide (F)		Concentration (C)	F x C
S. Em.±	0.35		0.23	0.61
C. D. at 5%	1.00		0.65	1.73
C.V.%	1.67			

*figures outside the parentheses are arcsine transformed, whereas inside are retransformed values

B. Efficacy of systemic fungicides

The data presented in Table 2, figure 2 and Plate 2 revealed that all systemic fungicides were capable in reducing the mycelial growth of test pathogen as compared to control. Among different systemic fungicides tried *in vitro*, propiconazole 25 EC found significantly superior over rest of the treatments with 92.76 percent mean mycelial growth inhibition. Thiophanate methyl 70 WP exhibited 38.11 percent mean mycelial growth inhibition. The interaction effect between systemic fungicides and concentrations showed that propiconazole 25 EC and tebuconazole 25.9 EC each at 500 ppm concentration remained equally effective and significantly superior over rest of the treatments with

94.11 percent mycelial growth inhibition of test pathogen. Whereas, minimum mycelial growth inhibition was observed in thiophanate methyl 70 WP (27.97%) at 100 ppm concentration.

The present findings were in close conformity with the results of Zade *et al.* (2018) ^[14], Rani *et al.* (2018) ^[10] and Kommata (2019) ^[8]. They reported propiconazole as the most effective treatment against *A. alternata* and carbendazim 50 WP and thiophanate methyl 70 WP as the least effective in controlling mycelial growth of *A. alternata* while working with Alternaria blight in soybean, pigeonpea and wheat, respectively.

Table 2: Efficacy of systemic fungicides against *A. alternata* *in vitro*

Systemic fungicides	Mycelial growth inhibition (%)			Mean mycelial growth inhibition (%)
	100 ppm	250 ppm	500 ppm	
Azoxystrobin 23 SC	45.45 (50.78)	48.75 (56.53)	57.75 (71.53)	50.65 (59.61)
Carbendazim 50 WP	37.39 (36.88)	38.49 (38.73)	45.55 (50.97)	40.48 (42.19)
Difenoconazole 25 EC	70.24 (88.57)	72.21 (90.67)	73.49 (91.92)	71.98 (90.39)
Tebuconazole 25.9 EC	70.09 (88.40)	72.89 (91.35)	75.95 (94.11)	72.98 (91.28)
Kresoxim-methyl 44.3 SC	45.98 (51.71)	46.72 (53.00)	52.54 (63.01)	48.41 (55.91)
Propiconazole 25 EC	73.08 (91.53)	74.26 (92.64)	75.95 (94.11)	74.43 (92.76)
Thiophanate methyl 70 WP	31.93 (27.97)	36.95 (36.13)	45.13 (50.23)	38.00 (38.11)
Mean	53.45 (62.26)	55.75 (65.58)	60.91 (73.70)	-
	Fungicide (F)		Concentration (C)	F x C
S. Em.±	0.31		0.20	0.53
C. D. at 5%	0.88		0.58	1.52
C.V.%	1.63			

*figures outside the parentheses are arcsine transformed, whereas inside are retransformed values

C. Efficacy of ready-mixed fungicides

The data presented in Table 3, Figure 3 and Plate 3 revealed that all ready-mixed fungicides were capable in reducing the

mycelial growth of test pathogen as compared to control. Among all ready-mixed fungicides tried *in vitro*, hexaconazole 4 + zineb 68 WP found significantly superior

over rest of the treatments with mean mycelial growth inhibition of 92.70 percent. But, it was remained statistically at par with tebuconazole 50 + trifloxystrobin 25 WG with 92.03 percent mean mycelial growth inhibition. The treatment kresoxim methyl 15 + chlorothalonil 56 WG showed minimum mean mycelial growth inhibition of 78.57 percent. The interaction effect between the ready-mixed fungicides and concentrations revealed that, tebuconazole 50 + trifloxystrobin 25 WG at 1000 ppm concentration proved significantly superior over rest of the treatments with 93.75 percent mycelial growth inhibition of *A. alternata* *in vitro*. But, it was remained statistically at par with hexaconazole 4 + zineb 68 WP at 1000 and 750 ppm concentration with 93.56 and 93.00 percent mycelial growth inhibition, respectively. Whereas, carbendazim 12 +

mancozeb 63 WP at 500 ppm concentration remained less effective as compared to other treatments and had minimum mycelial growth inhibition of 73.57 percent. But, it was remained statistically at par with kresoxim methyl 15 + chlorothalonil 56 WG at the same concentration with 74.50 percent mycelial growth inhibition.

More or less similar type of trend using ready-mixed fungicides were also reported by different scientists while working with *Alternaria* spp. The effectiveness of hexaconazole 4 + zineb 68 WP and tebuconazole 50 + trifloxystrobin 25 WG against *A. alternata* in wheat and marigold had been reported by Kommata (2019) [8] and Jarwar *et al.* (2021) [6], respectively. Which support the results of present findings.

Table 3: Efficacy of ready-mixed fungicides against *A. alternata* *in vitro*

Ready-mixed fungicides	Mycelial growth inhibition (%)			Mean mycelial growth inhibition (%)
	500 ppm	750 ppm	1000 ppm	
Azoxystrobin 18.2 + Difenconazole 11.4 SC	72.70 (91.15)	73.08 (91.53)	73.66 (92.09)	73.15 (91.59)
Metiram 55 + Pyraclostrobin 5 WG	64.96 (82.09)	66.67 (84.31)	67.56 (85.43)	66.40 (83.95)
Carbendazim 12 + Mancozeb 63 WP	59.06 (73.57)	62.55 (78.76)	71.97 (90.42)	64.53 (80.92)
Fluxapyroxad 25 + Pyraclostrobin 25 SC	71.27 (89.69)	72.14 (90.60)	73.86 (92.28)	72.43 (90.85)
Hexaconazole 4 + Zineb 68 WP	73.08 (91.53)	74.66 (93.00)	75.30 (93.56)	74.35 (92.70)
Kresoxim methyl 15 + Chlorothalonil 56 WG	59.67 (74.50)	62.94 (79.31)	64.83 (81.91)	62.48 (78.57)
Tebuconazole 50 + Trifloxystrobin 25 WG	71.97 (90.42)	73.49 (91.92)	75.53 (93.75)	73.66 (92.03)
Mean	67.53 (84.71)	69.36 (87.06)	71.82 (89.92)	-
	Fungicide (F)		Concentration (C)	F x C
S. Em.±	0.30		0.19	0.52
C. D. at 5%	0.85		0.56	1.47
C.V.%	1.28			

*figures outside the parentheses are arcsine transformed, whereas inside are retransformed values

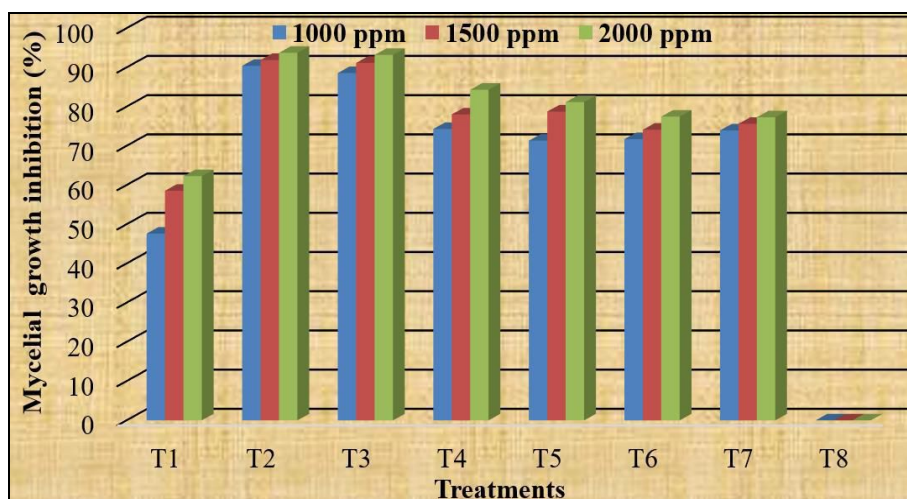


Fig 1: Efficacy of non-systemic fungicides against *A. alternata* *in vitro*

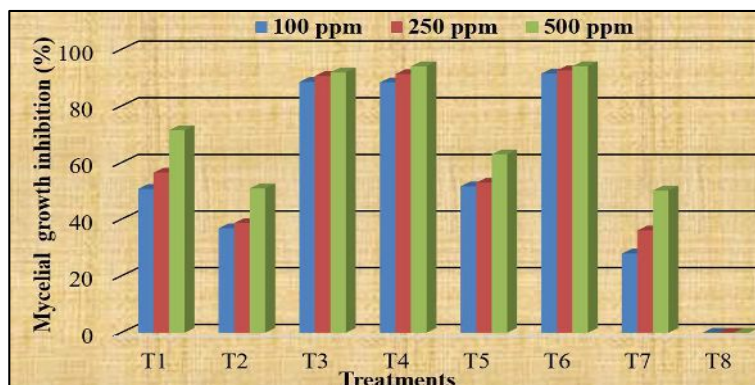


Fig 2: Efficacy of systemic fungicides against *A. alternata* in vitro

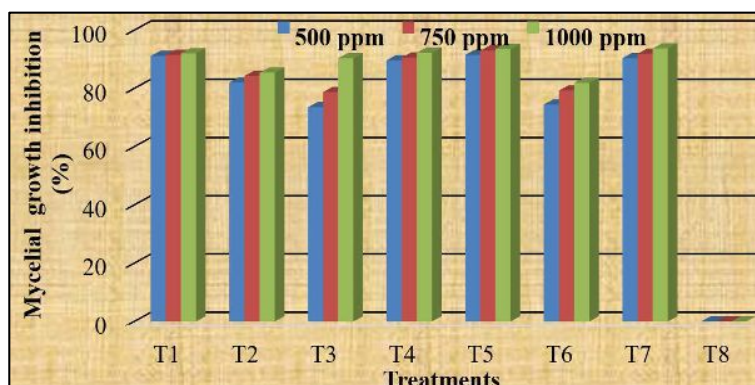


Fig 3: Efficacy of ready-mixed fungicides against *A. alternata* in vitro

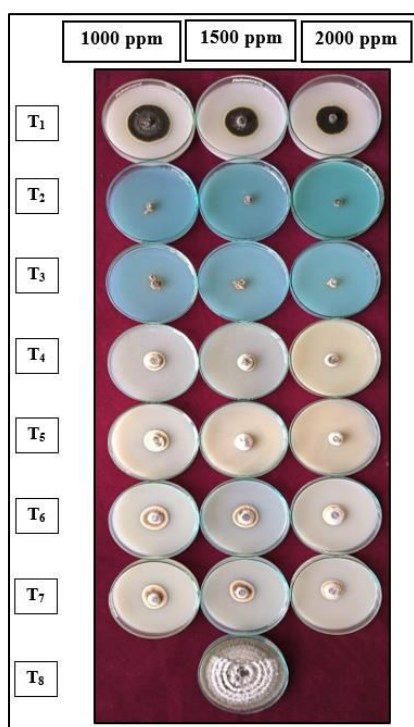


Plate 1: Efficacy of non-systemic fungicides against *A. alternata* in vitro

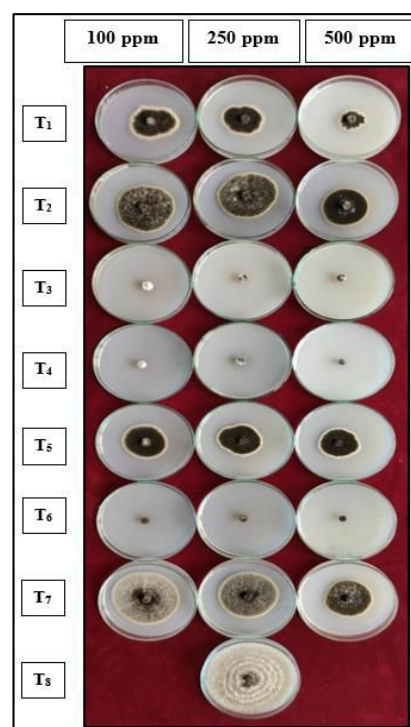


Plate 2: Efficacy of systemic fungicides against *A. alternata* in vitro

Treatments

T₁: Chlorothalonil 75 WP
 T₂: Copper hydroxide 53.8 DF
 T₃: Copper oxychloride 50 WP
 T₄: Mancozeb 75 WP
 T₅: Metiram 70 WG
 T₆: Propineb 70 WP
 T₇: Zineb 75 WP
 T₈: Control

Treatments

T₁: Azoxystrobin 23 SC
 T₂: Carbendazim 50 WP
 T₃: Difenconazole 25 EC
 T₄: Tebuconazole 25.9 EC
 T₅: Kresoxim methyl 44.3 SC
 T₆: Propiconazole 25 EC
 T₇: Thiophanate methyl 70 WP
 T₈: Control

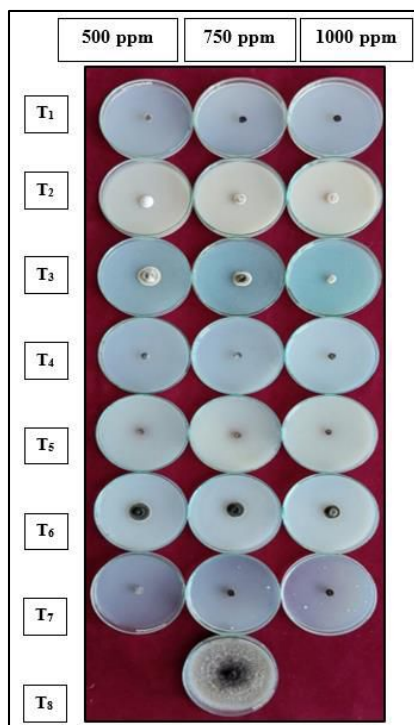


Plate 3: Efficacy of ready-mixed fungicides against *A. alternata* in vitro

Treatments

- T₁: Azoxystrobin 18.2 + Difenconazole 11.4 SC
 T₂: Metiram 55 + Pyraclostrobin 5 WG
 T₃: Carbendazim 25 + Mancozeb 50 WS
 T₄: Fluxapyroxad 25 + Pyraclostrobin 25 SC
 T₅: Hexaconazole 4 + Zineb 68 WP
 T₆: Kresoxim methyl 15 + Chlorothalonil 56 WG
 T₇: Tebuconazole 50 + Trifloxystrobin 25 WG
 T₈: Control

Conclusion

Based on the present study, it can be concluded that copper hydroxide 53.8 DF among non-systemic fungicides at 2000 ppm concentration; propiconazole 25 EC and tebuconazole 25.9 EC each at 500 ppm concentration among systemic fungicides and tebuconazole 50 + trifloxystrobin 25 WG at 1000 ppm and hexaconazole 4 + zineb 68 WP at 1000 and 750 ppm concentrations among ready-mixed fungicides gave maximum percent mycelial growth inhibition of *A. alternata* in vitro.

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Conflict of interest. None.

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