



ISSN (E): 2277-7695

ISSN (P): 2349-8242

NAAS Rating: 5.23

TPI 2023; 12(1): 01-05

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www.thepharmajournal.com

Received: 01-10-2022

Accepted: 07-11-2022

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In vitro efficacy of fungicides against *Alternaria cucumerina*, causing leaf blight of bottle gourd

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DOI: <https://doi.org/10.22271/tpi.2023.v12.i1a.17891>

Abstract

Alternaria cucumerina (Ellis and Everh.) Elliott leaf blight is one of the threats limiting to bottle gourd (*Lagenaria siceraria*) cultivation in India. Considering the economic importance of the crop as well as destructive nature of the disease, present *in vitro* studies were undertaken to evaluate the efficacy of seven each systemic fungicides (each @ 500 and 1000 ppm) and non-systemic/combi-product fungicides (each @ 2000 ppm and 2500 ppm), to assess their potential against *Alternaria cucumerina*. Results indicated that among systemic fungicides mean mycelial growth inhibition recorded with the test systemic fungicides ranged from 63.89 to 100 per cent. However, the significantly highest and cent per cent (100%) inhibition was with Propiconazole 25% EC and Tebuconazole 25.9% EC, followed by Hexaconazole 5% SC (96.36%). Among non-systemic/combi-product fungicides highest mean mycelial growth inhibition was recorded with Carbendazim 25% + Mancozeb 50% WP (96.11%), followed by Mancozeb 75% WP (95.83%) and Chlorothalonil 75% WP (91.11%).

Keywords: *Lagenaria siceraria*, *Alternaria cucumerina*, fungicides, systemic, non-systemic, inhibition

Introduction

Bottle gourd (*Lagenaria siceraria*) is one of the most important cucurbitaceous vegetable crop being grown round the year in various parts of India and the major states are Maharashtra, U.P, Bihar, West Bengal, Assam, Punjab and Gujarat. In India, the area under bottle gourd cultivation was 192 thousand ha., with production of 3143 thousand MT and productivity of 16.36 tonnes/ha. Whereas, in Maharashtra area under cultivation was 3.02 thousand ha., production of 57.16 thousand MT and productivity of 18.92 tonnes/ha (Anon, 2021) ^[1].

It is gaining importance due to its high yield potential, nutritional values and steady market price throughout the season. However, bottle gourd is affected by a number of diseases viz., Powdery mildew (*Erysiphe cichoracearum*), Anthracnose (*Colletotrichum lagenarium*), *Alternaria* leaf blight (*Alternaria cucumerina*), Damping off (*Pythium* sp.) Downy mildew (*Pseudoperonospora cubensis*), Phytophthora crown and root rot (*Phytophthora capsici*), Cercospora leaf spot (*Cercospora citrullina*), Fusarium wilt (*Fusarium oxysporum* f. sp. *cucumerinum*) and Cucumber Mosaic virus, causing huge economic losses worldwide (Zitter *et al.*, 1998 and Maheshwari *et al.*, 2017) ^[14, 9].

Among these diseases, *Alternaria cucumerina* (Ellis and Everh.) Elliott leaf blight is one of the threat limiting to bottle gourd cultivation in India. The disease has been reported to cause 50-100 per cent yield losses in cucurbits, including bottle gourd (Watt, 2013; Maheshwari *et al.*, 2017) ^[13, 9]. Considering economic importance of the crop as well as destructive nature of the disease, present *in vitro* studies were undertaken to evaluate the bioefficacy of seven each systemic fungicides and non-systemic/combi-product fungicides, to assess their potential against *A. cucumerina*, during 2020-21, at the Department of Plant Pathology, College of Agriculture, Latur.

Materials and Methods

Seven systemic (each @ 500, 1000 ppm), three non-systemic and four combi-product (each @ 2000, 2500 ppm) fungicides were evaluated *in vitro* against *A. cucumerina*, using PDA as basal culture medium and by applying Poisoned food technique (Nene and Thapliyal, 1993) ^[10]. Based on the active ingredient, requisite quantity of all the test fungicides was calculated, dispensed separately and mixed thoroughly with autoclaved and cooled (45 °C) PDA medium, in glass conical flasks (250 ml capacity) and prepared their desired concentrations. This PDA medium separately amended with the test fungicides was then poured (20 ml/plate) aseptically in sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature.

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For each test fungicide and its test concentration, three PDA plates per treatment per replication were maintained and replicated thrice.

After solidification of the fungicides amended PDA medium, all these plates were inoculated aseptically by placing in the centre a 5 mm culture disc of the test fungus, obtained from actively growing 7 days aged pure culture of the test pathogen. PDA plates without fungicide and inoculated with pure culture disc of the test pathogen were maintained as untreated control. Both treated and untreated PDA plates were incubated in an inverted position in BOD incubator (27 ± 2 °C). The experimental details were as given below.

Systemic fungicides

Tr. No.	Treatments	Tr. No.	Treatments
T1	Carbendazim 50% WP	T5	Tebuconazole 25.9% EC
T2	Hexaconazole 5% EC	T6	Azoxystrobin 23% SC
T3	Thiophanate methyl 70% WP	T7	Difenconazole 25% EC
T4	Propiconazole 25% EC	T8	Control (untreated)

Non-systemic and Combi-product fungicides

Tr. No.	Treatments	Tr. No.	Treatments
T1	Propineb 70% WP	T5	Azoxystrobin 18.2% + Difenconazole 11.4% SC
T2	Mancozeb 75% WP	T6	Tebuconazole 67% + Captan 26.9% SC
T3	Chlorothalonil 75% WP	T7	Azoxystrobin 11.00% + Tebuconazole 18.3% SC
T4	Carbendazim 25% + Mancozeb 50% WP	T8	Control (untreated)

Observations on radial mycelial growth/colony diameter (mm) of the test pathogen, at an interval of 24 hrs. of incubation were recorded and continued upto seven days or till the untreated PDA plates were fully covered with mycelial growth of the test pathogen. Based on cumulative data, per cent mycelial growth inhibition of the test pathogen with the test fungicides, over untreated control was calculated by applying the following formula (Vincent, 1927) [12].

$$\text{Per cent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth (mm) of the test fungus in untreated control plates.

T = Growth (mm) of the test fungus in treated plates.

Results and Discussion

The results obtained on *in vitro* efficacy of the test fungicides (systemic, contact and combi-product) against *A. cucumerina*, causing bottle gourd leaf blight are being narrated and discussed herein under the following sub-heads.

1. *In vitro* evaluation of systemic fungicides

All seven systemic fungicides evaluated *in vitro* (each @ 500, 1000 ppm) exhibited antifungal activity against *A. cucumerina* and significantly inhibited its mycelial growth over untreated control (Table 1, Plate 1, Fig. 1).

Table 1: *In vitro* efficacy of systemic fungicides against *A. cucumerina*, causing leaf blight of bottle gourd

Tr. No.	Treatments	Col. Dia.* (mm)		Avg. (mm)	% Inhibition		Avg. (%)
		At ppm			At ppm		
		500	1000		500	1000	
T ₁	Carbendazim 50% WP	43.50	21.50	32.50	51.67 (45.95)	76.11 (60.73)	63.89 (53.06)
T ₂	Hexaconazole 5% SC	6.50	0.00	3.25	92.78 (74.41)	100 (90.00)	96.39 (79.04)
T ₃	Thiophanate methyl 70% WP	32.50	23.50	28.00	63.89 (53.06)	73.89 (59.27)	68.89 (56.09)
T ₄	Propiconazole 25% EC	00	00	00	100 (90.00)	100 (90.00)	100 (90.00)
T ₅	Tebuconazole 25.9% EC	00	00	00	100 (90.00)	100 (90.00)	100 (90.00)
T ₆	Azoxystrobin 23% SC	20.50	19.0	19.75	77.22 (61.49)	78.90 (62.65)	78.06 (62.06)
T ₇	Difenconazole 25% EC	16.50	13.50	15.00	81.67 (64.65)	85.00 (67.21)	83.33 (65.90)
T ₈	Control (untreated)	90.0	90.0	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	S.E ±	0.74	0.62	-	0.67	0.58	-
	C.D. (P=0.01)	2.28	1.92	-	2.05	1.78	-

*Mean of three replications. Figures in parentheses are arcsine transformed values.

Diam.: Diameter, Av.: Average



A. @ 500 ppm

B. @ 1000 ppm

Plate 1: *In vitro* efficacy of systemic fungicides against *A. cucumerina*, causing leaf blight of bottle gourd

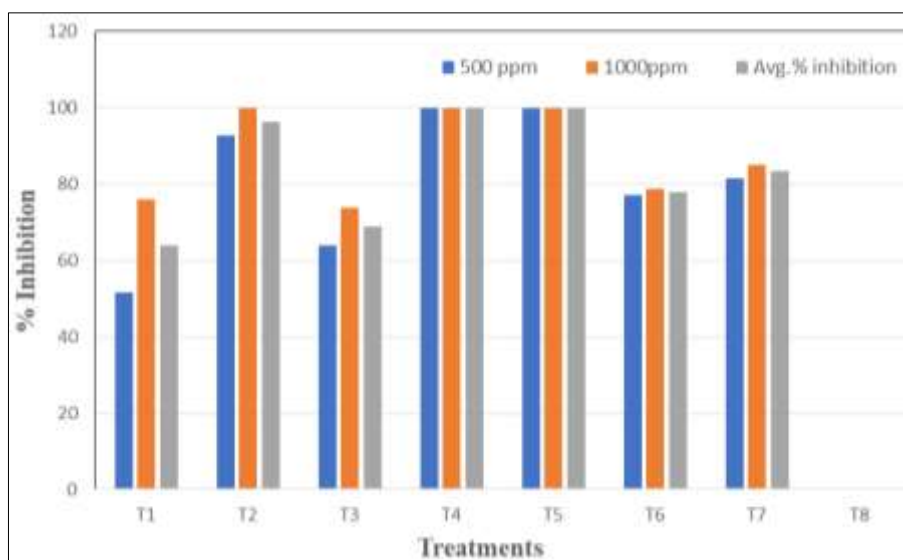


Fig 1: *In vitro* efficacy of systemic fungicides against *A. cucumerina*, causing leaf blight of bottle gourd

1.1 Effect on mycelial growth

Result (Table 1, Plate 1, Fig. 1) revealed that the test systemic fungicides exhibited a wide range of radial mycelial growth of *A. cucumerina*, which was found to be decreased drastically with increase in concentrations of systemic fungicides.

At 500 ppm, radial mycelial growth of *A. cucumerina* ranged from 00.00 to 43.50 mm. However, Propiconazole 25% EC and Tebuconazole 25.9% EC showed none of the growth of test pathogen. These were followed by Hexaconazole 5% SC (37.00 mm), Difenconazole 25% EC (16.50 mm), Azoxystrobin 23% SC (20.50 mm), Thiophanate methyl 70% WP (32.50 mm), Carbendazim 50% WP (43.50 mm).

At 1000 ppm, radial mycelial growth of *A. cucumerina* ranged from 00.00 to and 23.50 mm. However, Hexaconazole 5% SC, Propiconazole 25% EC and Tebuconazole 25.9% EC showed none of the growth of test pathogen. These were followed by Difenconazole 25% EC (13.50 mm), Azoxystrobin 23% SC (19.50 mm), Carbendazim 50% WP (21.50 mm) and Thiophanate methyl 70% WP (23.50 mm).

Average radial mycelial growth recorded with the test systemic fungicides, ranged from 00.00 mm to 32.50 mm. However, it was least with Hexaconazole 5% SC (3.25 mm), followed by Difenconazole 25% EC (15.00 mm), Azoxystrobin 23% SC (19.75 mm), Thiophanate methyl 70% WP (28.00 mm) and Carbendazim 50% WP (32.50), as against 90.00 mm in untreated control.

1.2 Effect on mycelial growth inhibition

The results (Table 1, Plate 1, Fig. 1) revealed that all the systemic fungicides tested (each @ 500 and 1000 ppm) significantly inhibited mycelial growth of *A. cucumerina*, over untreated control and it was directly proportional to concentrations of the test fungicides.

At 500 ppm, mycelial growth inhibition of *A. cucumerina* ranged from 51.67 to 100 per cent. However, Propiconazole 25% EC and Tebuconazole 25.9% EC showed cent per cent inhibition (100%). These were followed by Hexaconazole 5%

SC (92.78%), Difenconazole 25% EC (81.67%), Azoxystrobin 23% SC (77.22%), Thiophanate methyl 70% WP (63.89%) and Carbendazim 50% WP (51.67%).

At 1000 ppm, mycelial growth inhibition of *A. cucumerina* ranged from 73.89 to 100 per cent. However, Hexaconazole 5% SC, Propiconazole 25% EC and Tebuconazole 25.9% EC showed cent per cent inhibition (100%). These were followed by Difenconazole 25% EC (85.00%), Azoxystrobin 23% SC (78.90%), Carbendazim 50% WP (76.11%) and Thiophanate methyl 70% WP (73.89%).

Average mycelial growth inhibition recorded with the test systemic fungicides ranged from 63.89 to 100 per cent. However, it was cent per cent (100%) with Propiconazole 25% EC and Tebuconazole 25.9% EC, followed by Hexaconazole 5% SC (96.36%) and Difenconazole 25% EC (83.33%).

These results are in conformity with the findings of several earlier workers. Gohel and Solanky (2012) [6] reported Propiconazole, Difenconazole and Hexaconazole (each @ 250, 500, 1000 ppm) as most effective with cent per cent mycelial growth inhibition (100%) of *A. alternata*, causing Chilli leaf spot and fruit rot. Kumar *et al.* (2013) [8] reported cent per cent mycelial growth inhibition (100%) of *A. alternata*, causing Chilli leaf spot with Carbendazim 50% WP @ 0.1%; Apet *et al.* (2014) [2] reported highest mean mycelial inhibition of *A. alternata*, causing Gerbera leaf spot, with Hexaconazole 5% EC (94.44%) and Carbendazim 50% WP (84.93%).

2. *In vitro* efficacy of non-systemic and combi-product fungicides against *A. cucumerina*, causing leaf blight of bottle gourd

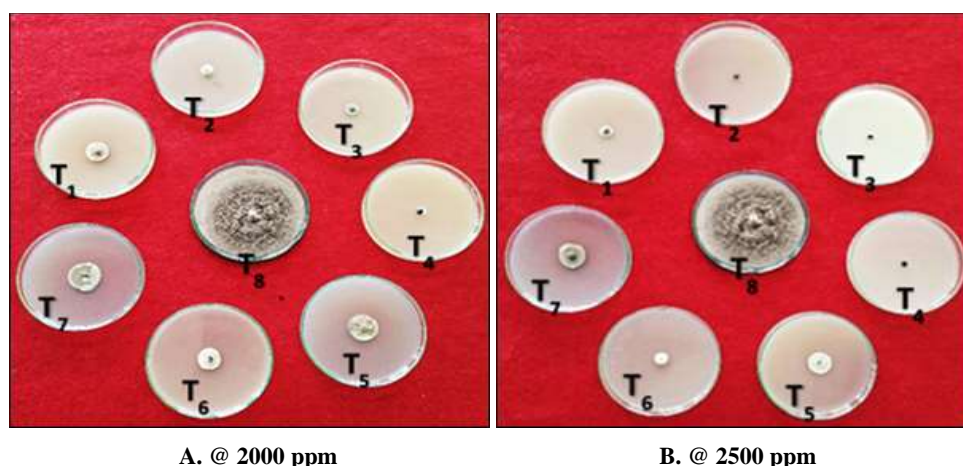
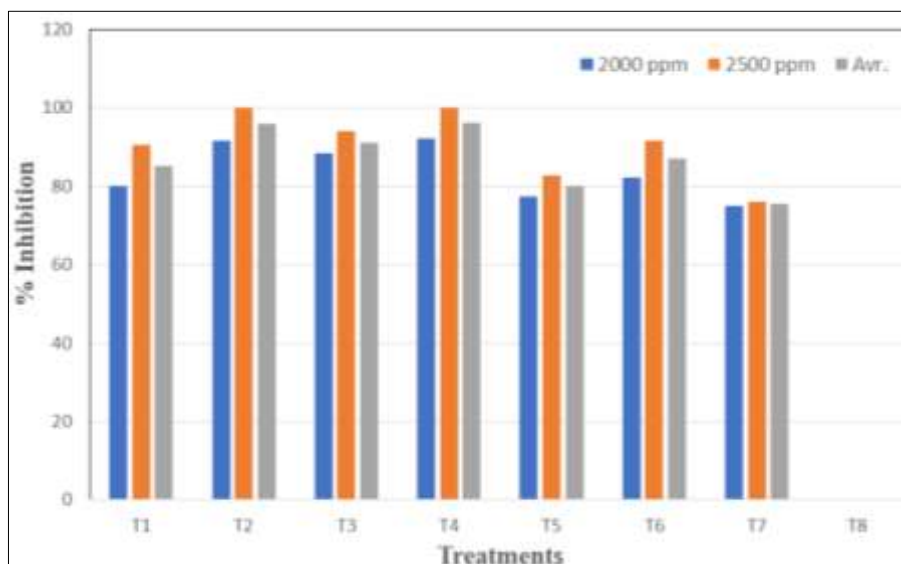
All of the seven non-systemic/combi-product fungicides evaluated *in vitro* (each @ 2000 and 2500 ppm) exhibited antifungal activity against *A. cucumerina* and resulted with significant mycelial growth inhibition, over untreated control (Table 2, Plate 2, Fig. 2).

Table 2: *In vitro* efficacy of non-systemic and combi-product fungicides against *A. cucumerina*, causing leaf blight of bottle gourd

Tr. No.	Treatments	Col. Dia.* (mm)		Avg. (mm)	% Inhibition		Avg. (%)
		At ppm			At ppm		
		2000	2500		2000	2500	
T ₁	Propineb 70% WP	18.00	8.50	13.25	80.00 (63.43)	90.55 (72.09)	85.27 (67.43)
T ₂	Mancozeb 75% WP	7.50	0.00	3.75	91.67 (73.22)	100 (90.00)	95.83 (78.21)
T ₃	Chlorothalonil 75% WP	10.50	5.50	8.00	88.33 (70.02)	93.89 (75.68)	91.11 (72.65)
T ₄	Carbendazim 25% + Mancozeb 50% WP	7.00	0.00	3.50	92.22 (73.80)	100 (90.00)	96.11 (78.62)
T ₅	Azoxystrobin 18.2% + Difenconazole 11.4% SC	20.50	15.50	18.00	77.22 (61.49)	82.78 (65.48)	80.00 (63.43)
T ₆	Tebuconazole 67% + Captan 26.9% SC	16.00	7.50	11.75	82.22 (65.06)	91.67 (73.22)	86.94 (68.81)
T ₇	Azoxystrobin 11.00% + Tebuconazole 18.3% SC	22.50	21.50	22.00	75.00 (60.00)	76.12 (60.74)	75.56 (60.37)
T ₈	Control (untreated)	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	S.E ±	0.86	0.71	-	0.73	0.69	-
	C.D. (P=0.01)	2.65	2.18	-	2.26	2.11	-

*: Mean of three replications, Figures in parentheses arcsine transformed values.

Diam.: Diameter, Av.: Average.

**Plate 2:** *In vitro* efficacy of non-systemic and combi-product fungicides against *A. cucumerina*, causing leaf blight of bottle gourd**Fig 2:** *In vitro* efficacy of non-systemic and combi-product fungicides against *A. cucumerina*, causing leaf blight of bottle

1.3 Effect on mycelial growth

Results (Table 2, Plate 2, Fig. 2) revealed that all the test non-systemic and combi-product fungicides exhibited a wide range of *A. cucumerina* mycelial growth, which was found to be decreased drastically with an increase in concentrations of the test fungicides.

At 2000 ppm, radial mycelial growth of *A. cucumerina* ranged from 7.00 to 22.50 mm. However, Carbendazim 25% + Mancozeb 50% WP resulted with minimum growth (7.00 mm), followed by Mancozeb 75% WP (7.50), Chlorothalonil

75% WP (10.50 mm), Tebuconazole 67% + Captan 26.9% SC (16.00 mm), Propineb 70% WP (18.00 mm), Azoxystrobin 18.2% + Difenconazole 11.4% SC (20.50 mm) and Azoxystrobin 11.00% + Tebuconazole 18.3% SC (22.50 mm). At 2500 ppm, radial mycelial growth of *A. cucumerina* ranged from 00.00 to and 21.50 mm. However, Mancozeb 75% WP and Carbendazim 25% + Mancozeb 50% WP showed none of the pathogen growth, followed by Chlorothalonil 75% WP (5.50 mm), Tebuconazole 67% + Captan 26.9% SC (7.50 mm), Propineb 70% WP (8.50 mm), Azoxystrobin 18.2% +

Difenconazole 11.4% SC (15.50 mm) and Azoxystrobin 11.00% + Tebuconazole 18.3% SC (21.50 mm).

Average radial mycelial growth recorded with the test non-systemic and combi-product fungicides ranged from 3.50 mm to 22.00 mm. However, it was least with Carbendazim 12% + Mancozeb 75% WP (3.50 mm), followed by Mancozeb 75% WP (3.75 mm) Chlorothalonil 75% WP (8.00 mm), Tebuconazole 67% + Captan 26.9% SC (11.75 mm), Propineb 70% WP (13.25 mm), Azoxystrobin 18.2% + Difenconazole 11.4% SC (18.00 mm) and Azoxystrobin 11.00% + Tebuconazole 18.3% SC (22.00 mm), as against 90.00 mm untreated control.

1.4 Effect on mycelial growth inhibition

Results (Table 2, Plate 2, Fig.2) revealed that all the non-systemic and combi-product fungicides tested (each @ 2000 and 2500 ppm) significantly inhibited mycelial growth of *A. cucumerina*, over untreated control and it was directly proportional to concentrations of the fungicides tested.

At 2000 ppm, mycelial growth inhibition of *A. cucumerina* ranged from 75.00 to 92.22 per cent. However, Carbendazim 12% + Mancozeb 75% WP resulted with significantly highest inhibition (92.22%), followed by Mancozeb 75% WP (91.67%), Chlorothalonil 75% WP (88.33%), Tebuconazole 18.3% SC + Captan 26.9% SC (82.22%), Propineb 70% WP (80.00%), Azoxystrobin 18.2% + Difenconazole 11.4% SC (77.22%) and Azoxystrobin 11.00% + Tebuconazole 18.3% SC (75.00%).

At 2500 ppm, mycelial growth inhibition of *A. cucumerina* ranged from 76.12 to 100 per cent. However, Mancozeb 75% WP and Carbendazim 25% + Mancozeb 50% WP resulted with cent per cent inhibition (100%), followed by Chlorothalonil 75% WP (93.89%), Tebuconazole 67% + Captan 26.9% SC (91.67%), Propineb 70% WP (90.55%), Azoxystrobin 18.2% + Difenconazole 11.4% SC (82.78%) and Azoxystrobin 11.00% + Tebuconazole 18.3% SC (76.12%).

Average mycelial growth inhibition recorded with the test non-systemic and combi-product fungicides ranged from 75.56 to 96.11 per cent. However, it was highest with Carbendazim 25% + Mancozeb 50% WP (96.11%), followed by Mancozeb 75% WP (95.83%), Chlorothalonil 75% WP (91.11%) and Tebuconazole 67% + Captan 26.9% SC (86.94%).

These results are in consonance with the findings of several earlier workers. Pawar *et al.*, (2014) ^[11] reported Mancozeb 75% WP @ 0.25% as most effective with cent per cent mean mycelial growth inhibition (100%) of *A. alternata*, causing Bottle gourd fruit rot. Apet *et al.* (2014) ^[2] reported Mancozeb 75% WP @ 1500 and 2500 ppm as most effective with 92.24% and 93.18% inhibition of *A. alternata*, causing Gerbera leaf spot. Hassan *et al.* (2014) reported significantly highest mycelial growth inhibition of *A. solani*, causing Tomato early blight with Chlorothalonil 75% WP @ 400 ppm (82.25%). Ginoya and Gohel (2015) ^[5] reported that Azoxystrobin 18.2% + Difenconazole 11.4% SC (@500, 1000 and 1500 ppm) as most effective with cent per cent mycelial growth inhibition (100.00%) of *A. alternata*, causing Chilli leaf spot). Ghazanfar *et al.* (2016) ^[4] reported highest mycelial inhibition of *A. solani*, causing Tomato early blight with Mancozeb 75% WP (62.29%) and Propineb (56.56%).

Conclusions

From ongoing results, it is concluded that Propiconazole 25% EC, Tebuconazole 25.9% EC, Hexaconazole 5% SC,

Difenconazole 25% EC (systemic); Mancozeb 75% WP (non-systemic); Carbendazim 12% + Mancozeb 63% WP, Carboxin 37.5% + Thiram 37.5% WP, Tebuconazole 67% + Captan 26.9% SC (combi-product) fungicides as most efficient in inhibiting mycelial growth of *A. cucumerina*, causing bottle gourd leaf blight. Thus, judicious use of these fungicides can be recommended to combat the leaf blight of bottle gourd.

Acknowledgement

I greatly acknowledge my research guide Dr. A.P. Suryawanshi, Ex. Professor, (Plant Pathology) and Former Associate Dean and Principal, College of Agriculture, Latur and my Advisory Committee Members, for their guidance and support to complete this research work, as a part of my M.Sc. degree programme.

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