International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(3): 4843-4848

Received: 27-03-2019
Accepted: 29-04-2019

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Study *in vitro* evaluation of plant extracts and fungicides against *A. cucumerina* var. *cyamopsidis*. of cluster bean

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Abstract
Culture media on growth of the fungus as well as colony characteristics of the isolated *Alternaria* from diseased Cluster bean leaves is needed to be developed for suitable management strategies of the disease and also knowledge of nutritional pattern and factors influencing the growth of the fungus. In *In vitro* bioassay of botanicals, total 15 treatments/botanicals including control were evaluated against *A. c.* var. *cyamopsidis in-vitro* conditions at @ 10% and 20% concentration. Maximum percentage inhibition was found using *Allium cepa* (75.41% and 100%), *P. hysterophorous* (68.73% and 100%) on both concentration after 7 days of inoculation while *C. fistula* showed the maximum inhibition after 3 days of inoculation. Minimum percentage inhibition was revealed by *Lantana camara* (31.83% and 45.38%) at both concentrations. *In vitro* bioassay of fungicides, total eleven treatments/fungicides including control were evaluated against *A.c.* var. *cyamopsidis in-vitro* conditions at 0.1 per cent and 0.2 per cent concentrations. Maximum percentage inhibition was found using Mancozeb (100% and 100%), Carbendazim (100% and 100%), Tebuconazole (91.72% 100%), Thiophanate methyl (100% and 100%), and Vitavax (60.32% and 85.76%) showed maximum inhibtion only after 3 and 5 days of inoculation. Minimum percentage inhibition was seen by Tricyclazole (32.96% and 55.91%) at both concentrations. The complete inhibition was found using *Allium cepa* and *P. hysterophorous* at 20% concentration under *in vitro* conditions. Complete inhibition was found using Mancozeb, Carbendazim, and Thiophanate methyl at 0.1 per cent concentration under *in vitro* conditions.

Keywords: Cluster bean (*Cyamopsis tetragonoloba* (L.), *Alternaria cucumerina* var. *cyamopsidis*

Introduction
Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] Belongs to the family Fabaceae. It is an important dry land, drought hardy, annual Kharif crop grow widely under rainfed (Barani) condition for grain, green fodder, vegetable, green manuring and for seed purposes. The major world suppliers are India, Pakistan and the United States with smaller acreages in Australia and Africa. Culture media on growth of the fungus as well as colony characteristics of the isolated *Alternaria* from diseased Cluster bean leaves is needed to be developed for suitable management strategies of the disease and also knowledge of nutritional pattern and factors influencing the growth of the fungus. The disease appears year after year in mild to severe form since the pathogen is seed- borne in nature (Sowell, 1965) [31]. In early stages of infection, the water soaked spots appear on leaf blade which later turn greyish to dark brown with concentric zonations, demarcated with light brown lines inside the spot on the under surface. The lesions are light to grayish brown. Higher yield losses (43-78%) were recorded when leaves were infected at seedling stage than at old stage (Sharma, 1981) [27]. For the assessment of nature of damage, caused by the pathogen, survey is essential. It also helps in identification of the specific pathogen species and its aggressiveness prevailing in a particular area. Although number of varieties with stable yield have been released which can be grown well under rainfed conditions but these varieties are susceptible to an array of phytopathogenic fungal and bacterial diseases. The production and productivity of Cluster bean in terms of grain and fodder is highly affected by a number of phytopathogenic fungal and bacterial diseases *viz*., bacterial blight (*Xanthomonas axonopodis* pv. *cyamopsidis*), *Alternaria* leaf spot (*Alternaria cucumerina* var. *cyamopsidis*), anthracnose (*Colletotrichum capsici* f. sp. *cyamopsicola*), Curvularia leaf spot (*Curvularia lunata*), charcoal rot/damping off (*Macrophomina phaseolina*), dry root rot/leaf blight *(Fusarium solani* and *Rhizoctonia solani*), Myrothecium leaf spot (*Myrothecium roridum*) and powdery mildew (*Oidiopsis taurica*).

Alternaria sps. are economically important pathogens widely distributed throughout the world and cause devastating disease on field crops. Alternaria leaf blight is a common disease in guar-growing area of western India and Pakistan. Severe Alternaria blight of cluster bean was also reported from Pusa and Madras (Ambesh *et al*., 2014) [13].

Materials and Methods

In the laboratory experiment, fourteen botanicals were evaluated against *A. c.* var. *cyamopsidis* by poisoned food technique. The standard crude extract and Potato dextrose agar (PDA) medium was mixed at required quantities to get 10 and 20 per cent concentrations. Seven mm culture discs of *A. c.* var. *cyamopsidis* isolated from guar leaves, were taken from the margin of seven days old colony and artificially placed in the centre of the Petri dish containing PDA medium. Plates without extract medium served as control. Three replications were maintained. The radial growth of the mycelium was measured after 7 days of incubation at 28±2 °C and per cent inhibition was calculated using the formula,

$$I\% = 100 \text{ X } (C- T)/C$$

Where,

I= Per cent inhibition of *A. c.* var. *cyamopsidids* growth
C= Linear growth in control (mm) and
T= Linear growth in treatment (mm)

The experiment was conducted in CRD with three replications. Following botanicals were evaluated against the growth of *A. c.* var. *cyamopsidis*.

Collection of botanicals

All the plant materials were collected from vicinity of College of Agriculture, Gwalior.

Preparation of plant extract

For the preparation of extract leaf/ bulb and rhizome were thoroughly washed in ordinary tap water. The leaves, rhizome and bulb were dried in air and cut into small pieces, then grinded and filtered (100mesh) and transferred into conical flasks for further use in investigations. The crude extract was used @ 10 ml and 20 ml solution of the respective botanical and incorporated into 90 ml and 80 ml PDA medium and pinch of streptomycin sulphate was mixed just before pouring respectively. The details of experiment are given below.

Evaluation of fungicides

For the evaluation of the fungicides ten systemic and one non systemic fungicides were used at 0.1 per cent and 0.2 per cent concentrations against *A. c.* var. *cyamopsidis* by Poison Food Technique. The required calculated quantity of fungicide was added to melted PDA medium, mixed thoroughly and poured into sterilized Petri plates and allowed to solidify. After solidification, each plate was inoculated with a 7 mm disc obtained from 7 days old actively growing culture of *A. c.* var. *cyamopsidis*. These Petri dishes were incubated at 28±2 °C. The data of efficacy of fungicides against *A. c.* var. *cyamopsidis* was recorded after 7th day of inoculation**.** The experiment was conducted in CRD with three replications. The inhibition percentage was calculated by measuring the mycelial growth in the fungicide amended petri plate and in control using the following

Results and Discussion

Total fifteen treatments were evaluated against *A. cyamopsidis* under *in-vitro* condition at the concentration of 10% and 20% and the data are summarized in Table 17. At 20% concentration two botanicals *viz.; Allium cepa* and *Parthenium hysterophorous* completely inhibited the mycelial growth at 3, 5 and 7 days after inoculation while the maximum growth was recorded in control. These two botanical extracts were found significantly superior over all the remaining thirteen treatments while *Cassia fistula* showed absolutely inhibition after 3 DAI. The maximum mycelium growth 47.9mm was seen with *Lantana camara* after 7 DAI which was followed *Bougainvillea* sp. (40.5mm), *Tagetes erecta* (40.4mm) (Figure 1 and Plate 1-2).

Table 1: *In vitro* evaluation of botanicals at different concentrations against *A. c.* var. *cyamopsidis*

Name of botanicals	10% Concentration		20% Concentration	
	Mycelial growth (mm) at 7 DAI	Percentage inhibition	Mycelial growth (mm) at 7 DAI	Percentage inhibition
Allium cepa	21.57 (27.66)	75.41	0.0 (0.0)	100.00
Azadirachta indica	35.90 (36.79)	59.07	27.4 (31.2)	69.33
Parthenium hysterophorus	27.43 (31.57)	68.73	0.0 (0.0)	100.00
Calotropis procera	50.03 (45.00)	42.97	29.3 (32.5)	66.93
Eucalyptus globulus	45.30 (42.28)	48.36	37.6 (37.8)	57.13
Cassia tora	59.50 (50.45)	32.17	39.3 (38.7)	55.23
Bougainvillea sp.	56.93 (48.96)	35.10	40.5 (39.5)	53.82
Tagetes erecta	58.37 (49.79)	33.46	40.4 (39.4)	53.90
Lantana camara	68.57 (55.87)	31.83	47.9 (43.7)	45.38
Allium sativum	39.67 (39.02)	54.78	30.9 (33.7)	64.80
Zingiber oficinale	40.40 (39.44)	53.94	33.8 (35.5)	61.50
Ocimum sanctum	49.03 (44.42)	44.11	34.4 (36.0)	60.43
Datura stramonium	37.30 (37.62)	57.48	34.7 (36.0)	60.47
Cassia fistula	20.20 (26.69)	76.97	10.4 (18.9)	87.95
Control	87.73 (69.47)	0.00	87.7 (69.4)	0.00
CD at 5%	0.51		0.56	
SE(m)	0.17		0.19	

The figure in Parthenogenesis () are the Angular transformed values

In vitro* evaluation of fungicides against *A. c.* var. *cyamopsidis

The efficacy of fungicides was evaluated *in vitro* at two concentrations viz., 0.1 per cent and 0.2 per cent concnetrations against *A. c.* var. *cyamopsidis* on PDA by poisoned food technique. The data presented in Table 18 showed that increase in concentration of the fungicides caused increased inhibition of mycelial growth of the fungus. Among these, Mancozeb, Carbendazim, and Thiophanate methyl completely inhibited the mycelial growth of *A. cyamopsidis* at both the concentrations whereas Tebuconazole completely inhibited the mycelial growth of the pathogen only at 0.2 per cent concentration. The fungicide Tricyclazole was found least effective at all concentrations (Figure 2, Plate 3-4).

Table 2: *In vitro* evaluation of fungicides at different concentrations against the growth of *A. c.* var. *cyamopsidis*

Fungicides	0.1 per cnet concentration		0.2 per cnet concentration	
	Mycelial growth (mm) at 7 DAI	Percentage inhibition	Mycelial growth (mm) at 7 DAI	Percentage inhibition
Mancozeb	0.00 (0.0)	100.00	0.00 (0.0)	100.00
Vitavax	34.40 (35.8)	60.32	12.27 (20.49)	85.76
Carbendazim	0.00 (0.0)	100.00	0.00 (0.0)	100.00
Hexaconazole	35.27 (36.4)	58.71	9.73 (18.17)	88.71
Kitazin	34.33 (35.8)	59.82	22.30 (28.16)	74.12
Tebuconazole	7.37 (15.7)	91.72	0.00 (0.0)	100.00
Propiconazole	18.27 (25.2)	78.61	12.13 (20.37)	85.92
Tricyclazole	59.20 (50.2)	32.96	38.00 (38.04)	55.91
Thiophanate methyl	0.00 (0.0)	100.00	0.00 (0.0)	100.00
Azoxystrobin	44.57 (41.8)	47.36	27.20 (31.42)	68.44
Control	85.33 (67.4)	0.00	86.20 (68.16)	0.00
CD at 5%	0.43		0.12	
SE(m)	0.14		0.04	

The value in parenthesis is transformed value.

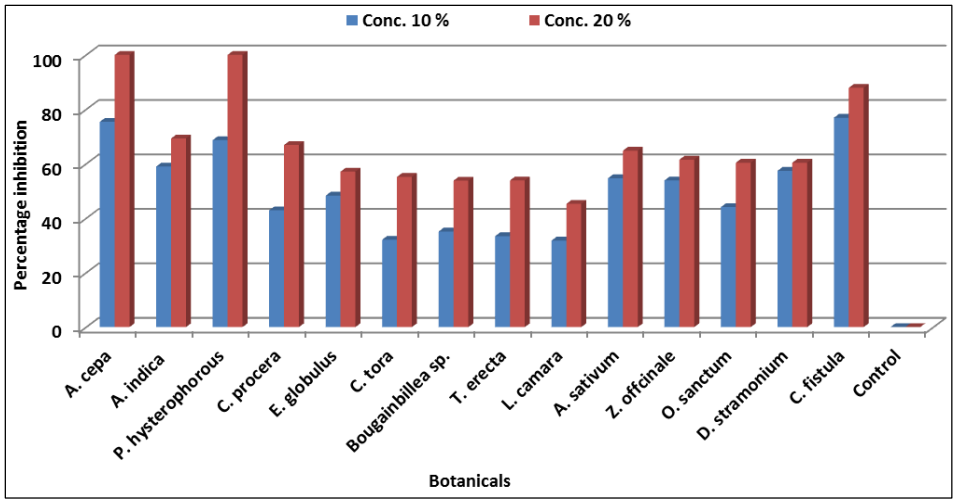

Fig 1: *In vitro* evaluation of botanicals at different concentrations against *A. c.* var. *cyamopsidis*

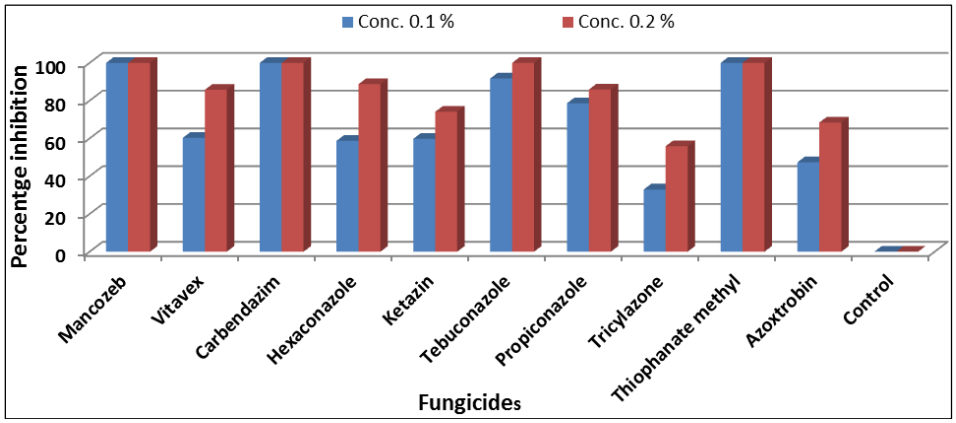

Fig 2: *In vitro* evaluation of fungicides at different concentrations against *A. c.* var. *cyamopsidis*

Plate 1: *In vitro* evaluation of botanicals against *A. c.* var. *cyamopsidis* at 10% concentration.

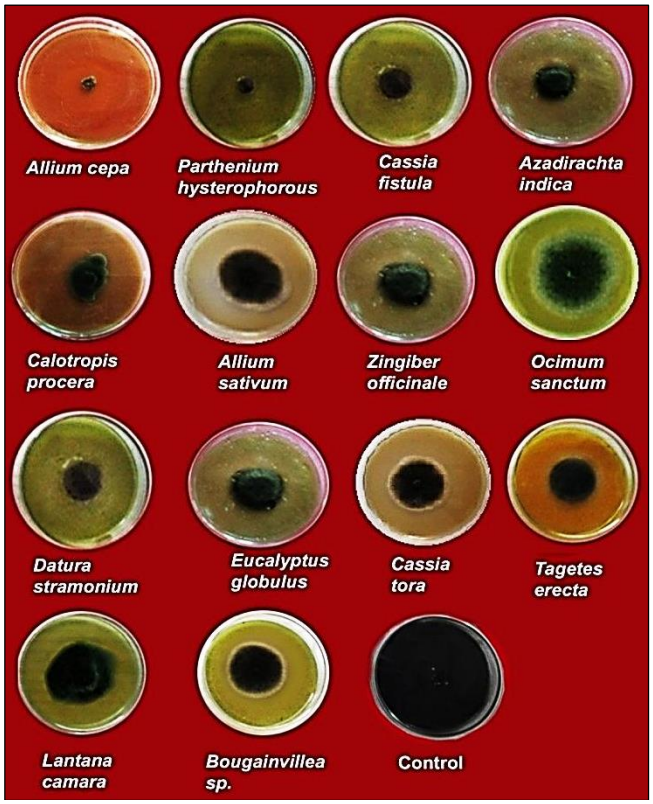

Plate 2: *In vitro* evaluation of plant extracts against *A. c.* var. *cyamopsidis* at 20% concentration

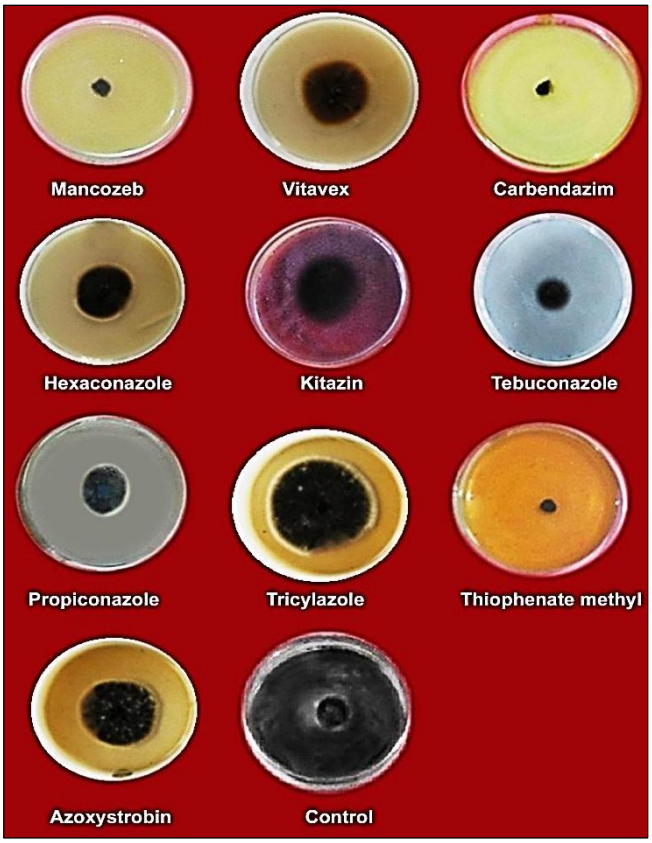

Plate 3: *In vitro* evaluation of fungicides against growth of *A. c.* var. *cyamopsidis* at 0.1 per cent concentration.

Plate 4: In vitro evaluation of fungicides against growth of Alternaria cucumerina var. cyamopsidis at 0.2 per cent concentration

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The possibilities of exploiting cultivars resistant in controlling guar disease are limited. The commercial cultivars either do not exhibit sufficient degree of resistant or the resistant is turned down by new physiological races. In the absence of commercially available resistant cultivars and at the time of possibility of epidemic situation use of fungicidal botanicals

is the only quick and immediate method for control of the disease.

The fungitoxicity of commonly available plant products viz., *Parthenium hysterophorus, Datura stramonium, Eucalyptus isobus, Azadirachta indica* and *Calotropis procera* were screened *in-vitro* and *in-vivo* against *Alternaria cyamopsidis* which causes a serious threat to cultivation. It was thought proper to explore and exploit the plant products for the control of plant diseases as indicated in the several reviews. A number of plant species have been reported to possess some natural substances in their leaves which were toxic to many fungi causing plant disease (Spencer *et al.*, 1957) [32]; Egawa *et al.*, 1977 [6]; Mishra and Dixit, 1976 [20], Shekhawat and Prasad, 1971 [28]; Tripathi and Dixit, 1981) [35]. Botanical extracts are biodegradable and their use in crop protection is a practical sustainable alternative. It reduces environmental contamination and health hazard (Grange and Ahamad, 1988) [9]. Botanical fungicides are unique because they can be produced easily by the farmers and small industries (Roy *et al*., 2005) [26].

Although spraying of fungicides are not common in the cultivators field but with the appearance of disease like Alternaria blight, it becomes obligatory to have a spraying schedule. The concept of organic farming and ecofriendly management has encouraged the plant protectionists to go for the use of plant extracts for the management of diseases. Also this can avoid the pollution of air, water and soil. In an attempt to check the growth of *A. c.* var. *cyamopsidis* fourteen botanicals were tested *in-vitro* by poisoned food technique. Under *in-vitro* evaluation the botanicals significantly inhibited the fungal growth of the pathogen at @10 and 20 per cent concentration. Out of fourteen botanicals, maximum percentage inhibition was found in *Allium cepa, P. hysterophorous* at both concentrations while *C. fistula* showed the maximum inhibition after 3 days of inoculation. Minimum percentage inhibition was revealed by *Lantana camara* at both concentrations.

The inhibitory action on mycelial growth might be due to the presence of inhibitory substances in the extract. Various workers reported to the presence of antifungal compounds in the plant extracts (Kumar and Sachan, 1979 [13]; Kumar *et al.*, 1998 [14]; Jagannathan and Narashinhan, 1988 [10]; Thangamani and Narayanaswamy, 1998 [34]; Godara and Pathak 1995 [8]; Arya *et al.*, 1994 [4]; Jeeva and Ramabadran, 1992 and Patil *et al.*, 1992) [11, 24]. The stimulatory effect is presumably due to the presence of same nutrients and stimulatory substances. Fungicide application can minimize disease and thus increase the genetic potential and ultimately yield. Therefore, it necessities the judicial use of fungicides at proper time. Eleven fungicides including control were evaluated *in vitro* against *A. c*. var. *cyamopsidis* at two concentrations @ 0.2% for non-systemic and @ 0.1% for systemic fungicieds by Poisoned food technique. All the tested fungicides significantly inhibited the growth of *A. cyamopsidis*. Maximum percentage inhibition was found with Mancozeb, Carbendazim, Tebuconazole and Thiophanate methyl whereas Tricyclazole showed minimum inhibition per cent.

The inhibitory action on mycelial growth might be due to the presence of inhibitory substances in the extract. Various workers reported to the presence of antifungal compounds in the plant extracts (Kumar and Sachan, 1979 [13]; Kumar *et al*., 1979 [13]; Jagannathan and Narashinhan, 1988 [10]; Thangamani and Narayanaswamy, 1998) [34]. In the present investigation, the maximum inhibition was recorded in Azoxystrobin and Tebuconazole. Similar finding were recorded by Ginoya and Gohe (2015) [7]. Among the ten newer fungicides evaluated under *in vitro* condition by poisoned food technique against *Alternaria alternata* revealed Tebuconazole, Hexaconazole and Azoxystrobin (18.2%) + Difenconazole (11.4%) at all the three concentrations (500, 1000 and 1500 ppm) completely inhibited the mycelial growth of the pathogen and proved to be most effective. Singh and Singh (2006) [29] tested efficacy of seven fungicides *viz.*; Chlorothalonil, Copper oxychloride, Azoxystrobin, Propineb, Copper hydroxide, Mancozeb at 2500, 2000, 1000, 500 and 250 ppm and Hexaconazole at 1000, 500, 200, 100 and 50 ppm against *A. alternata* causing blight of tomato. Their observations revealed that all the fungicides significantly reduced the radial growth of the fungus. The results obtained in present studies in respect of *in vitro* effect of fungicides on mycelial growth inhibition of the test pathogen for Azoxystrobin, Mancozeb, Propiconazole and Hexaconazole fungicides effect is similar with earlier workers (Amaresh and Nargund, 2004 [2]; Akbari and Parakhia, 2007 [1]; Mathivanan and Prabavathy, 2007) [17]. The present investigation revealed that least inhibition was recorded in Tricyclazole. It is supported by findings of Rakesh *et al*., (2016) [25] who observed that Tricyclazole failed to inhibit mycelial growth up to 100 ppm. Least inhibition of mycelial growth was observed in Beam 75WP (64.07%) at 500 ppm and it was not effective in reduction of growth of the fungus at 100 ppm (17.04%) (Nguyen, 2013) [22].

The study revealed that the fungicide Mancozeb significantly reduced the mycelial growth of the fungus. It was supported by Thaware *et al*., (2010) [33]. Among different fungicides tested *in vitro*, Mancozeb (0.2 per cent) and Propiconazole (0.05 per cent) completely inhibited the growth of the test fungus. Similar reports were also given by Dubey *et al*., (2000) [5]; Singh and Singh (2004) [30]. Propiconazole and Hexaconazole inhbited the growth of the *A. cyamopsidis*. It was supported by the results of Mane *et al*., (2011) [16] who reported cent per cent inhibition of radial growth with propiconazole, Dithane M-45 and Copper hydroxide, while Difenconazole and Hexaconazole were best among triazole group fungicides in inhibiting the radial growth of *A. alternata*. Similarly Patel (2008) [23], Akbari and Parakhia, (2007) [1] reported Hexaconazole, Penconazole, Propiconazole and compound fungicide *i.e.* Hexaconazole (5%) + Captan (70%) were highly fungitoxic against *A. alte*

Figure 2: *In vitro* evaluation of fungicides at different concentrations against *A. c.* var. *cyamopsidis*

rnata and cent per cent growth inhibition of *A. alternata* was noted at all three concentrations over control.

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