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Efficacy of fungicides against *Alternaria cucumeria* var. *cymopsidis* causing leaf spot disease of cluster bean under *in vitro* condition

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Abstract

Cluster bean is being grown in India since ancient times not only for consumption but also as a good source of nutrition and industrial prospectus. Its production suffers with many biotic and abiotic factors. Leaf blight spot disease triggered by *Alternaria cucumeria* var. *cymopsidis* is one of the frightful diseases in cluster bean causing enormous economic losses. The present study was conducted in order to evaluate the efficacy of different twelve fungicides against *Alternaria cucumeria*. The efficacy of these fungicides recorded at *in vitro* against *Alternaria cucumeria* at recommended doses 500 ppm, 1000 ppm, 1500 ppm. It was concluded that among all twelve fungicides used for this study, Tricyclazole, and Tebuconazole + Trifloxystrobin found to be superior at experimented doses with maximum inhibition of mycelium growth (100%).

Keywords: Cluster bean, fungicides, leaf spot

Introduction

Clusterbean [*Cyamopsis tetragonaloba* (L.) Taub] commonly known as 'guar' means "cow food" is an annual arid and semi-arid legume crop belonging to the family leguminaceae. It is drought tolerant crop suitable for cultivation under rainfed conditions of kharif season in India. India produces about 80% of global cluster bean followed by Pakistan (15%), Sudan, Australia, and USA (Swamy *et al.*, 2015) [1]. It is also grown for production of seeds and extraction of guar gum purposes in India. Galacto2mannan (gum) is extensively used in textile, paper, petroleum, pharmaceuticals, food processing, cosmetics, mining, explosive oil drilling, photography etc. The guar gum is also known to have medicinal value in the treatment of diabetes and high cholesterol (Hymowitz, 1972) [2]. The green pods of cluster bean serve as a nutritious vegetable which contains 82.5% water, 3.7% protein, 9.9% carbohydrate, 0.2% fat, 2.3% fiber, 1.4% other minerals *viz.* 0.13% Calcium, 0.25% Phosphorus, 5.8mg/ 100g Iron and 49 mg/ 100g Vitamin (Anonymous, 2017-18) [3].

Among the biotic stress of *Alternaria* leaf of cluster bean and the causal agent is *Alternaria cucumeria* var. *cymopsidis*. *Alternaria* sps. are economically important pathogens widely distributed throughout the world and cause devastating disease on field crops (Abd El-Ghany, 2015) [4]. *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 (Arun, 2008) [5]. *Alternaria* infected the plant at all growth stages. Fungus infect all parts of plant as leaves, pods, branches, pods and stem but the special target point of fungus are leaves and pods. Often lesions are produced on green leaves and during sever attack in pods seeds become shrivel and early ripening or shattering. Based on the importance of *Alternaria* and its regular occurrence, the present investigation was carried out with a view to find out the efficacy of fungicides against *Alternaria cucumeria* var. *cymopsidis*.

Materials and Methods

Isolation Cluster bean leaves showing typical symptoms of disease were collected, washed with tap water to remove dirt, air dried and affected parts were cut into small pieces of about 2 cm in length. These pieces were disinfected with 1:1000 mercuric chloride solutions for a minute and rinsed with three changes of sterilized water to remove traces of corrosive disinfectant. These pieces were then transferred aseptically to sterilized plates poured with potato dextrose agar medium. The plates poured with sterilized medium were then incubated in inverted fashion in an incubator at 27 °C±1 temperature.

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Profuse growth of the fungus on plates was observed after one week. Following hyphal-tip technique, test pathogen was transferred aseptically on the PDA slant in test tubes. Through frequent sub-culturing, the pathogen was purified and its pure culture was maintained on agar slant in test tubes and stored in refrigerator for further studies.

Identification

Pure culture of test pathogen obtained was inoculated aseptically on autoclaved PDA in Petri plates and plates were incubated at 27±1 °C. A profuse growth of a fungus in plates was observed after one week. Cultural, morphological and microscopic characteristics of fully developed test pathogen were studied under low power as well as high power magnification of microscope.

Pathogenicity test

Ten days old culture of the organism was used for proving the pathogenicity by applying Koch's postulates. For this purpose, seeds of clusterbean GR-1(IVT+AVT2020) which is susceptible to *Alternaria* blight (*A. Cucumeria var. cymopsidis*) were surface sterilized with 0.1% HgCl₂, and sown in the earthen pots filled with steam sterilized potting mixture of soil:sand:FYM (2:1:1). Healthy growing clusterbean seedlings were maintained, watered regularly and kept in the screen house for further development. Three weeks old healthy seedlings were selected for inoculation. The spore suspension was prepared and filtered through two layers of sterile muslin cloth to remove residual mycelia. Filtrate obtained was suitably diluted with sterile distilled water to get inoculum concentration of 1×10⁶spores/ml. The seedlings were inoculated with 10 days old test fungus. Uninoculated seedlings of the same age sprayed with sterilized water served as control. After inoculation, the seedlings pots (both inoculated and uninoculated) were incubated in the screen house, where relative humidity (80 to 90%) and optimum temperature (27±1 °C) were maintained for further

development of *Alternaria* blight symptoms. Re-isolation was made from inoculated leaves by the isolated fungus which resembled in all respect with the original culture used for inoculation (Dean *et al.*, 2012) [6].

Efficacy of fungicides, (in vitro)

In vitro evaluation of fungicides

Efficacy of 12 fungicides viz., SAAF (Mancozeb 63% + arbandazim 12%) 75 WP, Mancozeb 75 WP, Chlorothalonil 75 WP, Hexaconazole 5 EC were evaluated *in vitro* against *A. cucumeria var. cymopsidis* by Poisoned food technique (Nene and Thapliyal, 1993). The requisite quantity of each fungicide based on active ingredient was calculated and mixed thoroughly with autoclaved and cooled (40°C) Potato dextrose agar medium (PDA) in conical flasks to obtain desired concentrations in ppm. Plain PDA medium without fungicide served as control. Fungicide amended PDA medium was then poured aseptically in Petri plates (90 mm dia.). After solidification of the medium, all the plates were inoculated aseptically with 5 mm culture disc of the test fungus obtained from a week old actively growing pure culture of *A. Cucumeria var. cymopsidis*. The disc was placed on PDA in inverted position in the centre of the Petri plates and plates were incubated at 27±1 °C. Each treatment was replicated thrice. When medium in the untreated control plates was fully covered with mycelial growth of the test fungus, radial mycelial growth was measured in all the treatment plates.

Per cent inhibition of mycelial growth in treated plates was calculated by applying the formula given by Vincent (1927).

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

Where,

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

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

Table 1: List of fungicides evaluated against *Alternaria* sp:

Treatment	Chemical name	Trade name	Rate of concentration
T1	Hexaconazole 5%EC	Contaf (TATA)	500 ppm, 1000 ppm 1500 ppm
T2	Carbendazim 50%WP	Zoom (UPL)	500 ppm, 1000 ppm 1500 ppm
T3	Carbendazim (12%)+ Mencozeb(63%)	SAAF	500 ppm, 1000 ppm 1500 ppm
T4	Copperoxychloride 50%WP	Blitox	500 ppm, 1000 ppm 1500 ppm
T5	Azoxystrobin 23%SC	Amistar	500 ppm, 1000 ppm 1500 ppm
T6	Mancozeb75%WP	Indofil	500 ppm, 1000 ppm 1500 ppm
T7	Propineb 70%WP	Antracol	500 ppm, 1000 ppm 1500 ppm
T8	Difenoconazole 25%EC	Score	500 ppm, 1000 ppm 1500 ppm
T9	Tricyclazole	Beam	500 ppm, 1000 ppm 1500 ppm
T10	Tebuconazole (50%)+ Trifloxystrobin (25%) WG	Nativo	500 ppm, 1000 ppm 1500 ppm
T11	Chlorothalonil (75%WP)	Kavach (Syngenta)	500 ppm, 1000 ppm 1500 ppm
T12	Metalaxyl (18%)+ Mancozeb(64%)	Mancodex super 72	500 ppm, 1000 ppm 1500 ppm

Result and Discussion

Evaluation of systemic and Nonsystemic fungicides against *Alternaria cucumeria var. cymopsidis* under *in vitro* conditions

All the twelve fungicides at three concentrations (500, 1000

and 1500 ppm) were tested significantly reduced radial growth of *Alternaria cucumeria var. cymopsidis*. (Table.2). Mycelial growth and its inhibition were found inversely and directly proportional, respectively to concentrations of the fungicides tested.

Table 1: Evaluation of systemic and Nonsystemic fungicides against *Alternaria cucumeria var.cymopsidis* under *in vitro* conditions:

Treatment No.	Treatments	Colony diameter (mm) at ppm			Percent inhibition (%)			Mean of percent inhibition
		500	1000	1500	500	1000	1500	
T1	Hexaconazole	17.92	15.65	8.53	70.09	72.61	80.52	74.40
T2	Carbendazim	67.88	54.55	20.68	14.57	29.39	67.02	36.99
T3	Carbendazim(12%)+ Mencozeb(63%)	5.63	3.4	2.45	83.74	86.22	87.28	85.75
T4	Copperoxychloride	10.86	12.1	5.85	77.93	76.56	83.5	79.33
T5	Azoxystrobin	24.18	19.18	0	63.13	68.69	100	77.27
T6	Mancozeb	41.33	38.3	13.66	44.08	47.44	74.82	55.45
T7	Propineb	68.17	33.03	12.3	14.26	53.3	76.33	47.96
T8	Difenoconazole	53.24	25.35	15.31	30.84	61.83	72.98	55.22
T9	Tricyclazole	0	0	0	100	100	100	100
T10	Tebuconazole+ Trifloxystrobin	0	0	0	100	100	100	100
T11	Chlorothalonil	68.55	38.46	16.06	13.83	47.26	72.15	44.42
T12	Metalaxyl+Mancozeb	19.62	10.66	5.47	68.2	78.15	83.92	76.76
T13	Control	90	90	90	0.00	0.00	0.00	0.00

C.D. 0.555, SE(m) 0.190, SE(d) 0.269, C.V. 1.713

Tricyclazole, and Tebuconazole + Trifloxystrobin @ 500, 1000 and 1500 ppm showed complete inhibition (100%) with no radial growth of mycelium whereas, the radial growth in Hexaconazole, Carbendazim, Carbendazim + Mancozeb, copperoxychloride, Azoxystrobin, Mancozeb, propineb Difenoconazole Chlorothalonil Metalaxy+mencozeb, and @ 500 were followed by 17.92mm 67.88 mm 5.63mm 10.86mm, 24.18 mm, 41.33 mm, 68.17 mm, 53.24 mm and 19.62 mm, 41.33 mm, 53.24 mm, 67.88 mm, 68.55 mm, and 19.62 mm with 70.09, 14.57, 83.74, 77.93, 63.13, 44.08, 14.26, 30.84,

13.83, and 68.2 per cent inhibition respectively. Hexaconazole, Carbendazim, Carbendazim + Mancozeb, copperoxychloride, Azoxystrobin, Mancozeb, propineb Difenoconazole Chlorothalonil Metalaxy+mencozeb, @ 1000 ppm treatment recorded 15.65 mm, 54.55 mm, 3.40, 12.10mm, 19.18mm, 38.30 mm, 33.03 mm, 25.35 mm, 38.46 mm and 10.66 mm radial growth respectively and its per cent inhibition were 72.61, 29.39, 86.22, 76.56, 68.69, 47.44, 53.3, 61.83, 47.26, and 78.15 per cent respectively as shown in figure 1 and 2.

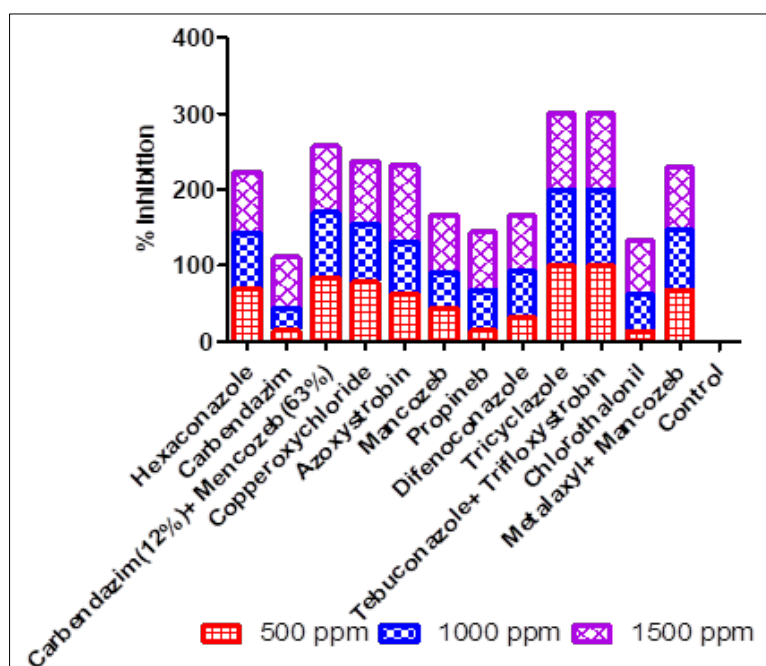


Fig 1: Effect of fungicides on per cent growth inhibition of *Alternaria macrospora in vitro*

Carbendazim+Mancozeb, copperoxychloride, Metalaxy+mencozeb, Mancozeb, Difenoconazole, Carbendazim, propineb and Chlorothalonil @ 1500 ppm treatment recorded 8.53 mm,

0.00 mm, 13.66 mm, 12.3 mm, 15.31 mm, 16.06 mm and 5.47 mm radial growth respectively and its per cent inhibition were 80.52, 67.02, 67.8, 87.28, 83.5, 100, 74.82, 76.33, 72.98, 72.15 and 83.92 per cent respectively.

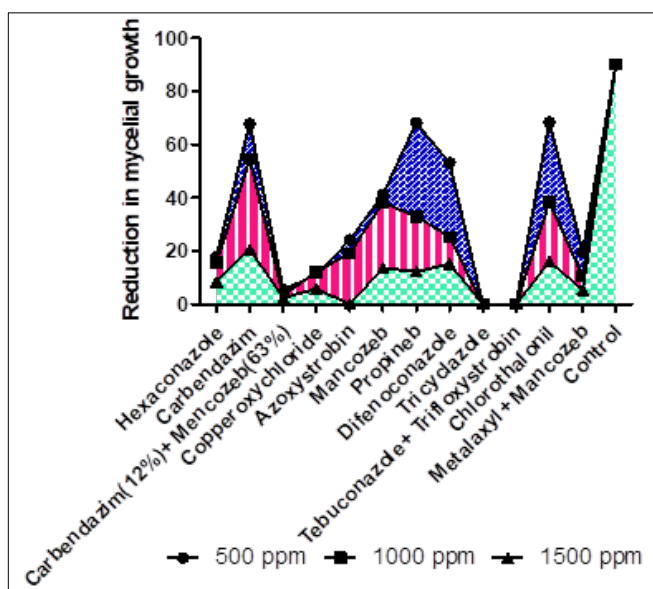


Fig 2: Effect of fungicides on mycelium growth of *Alternaria macrospora* in vitro

Most effective fungicides @ 500ppm Carbendazim + Mancozeb (83.74%), Copperoxychloride (77.93%), Hexaconazole (70.29%) and Metalaxyl + Mancozeb (68.2%), Azoxystrobin (63.2%). Most effective fungicides @1000ppm Carbendazim+Mancozeb (86.22%), Metalaxy+mencozeb (78.15%), copperoxychloride (76.56%) Hexaconazole (72.16%), Azoxystrobin (68.69%), and Difenconazole (61.83%). Most effective fungicides @ 1500ppm Carbendazim+Mancozeb (87.28%) copperoxychloride (83.5%), Hexaconazole (80.52%), Propineb (76.33%), Mancozeb (72.98%), and Carbendazim (67.02%).

Least effective fungicides recorded @ 500ppm Chlorothalonil (13.83%), propineb (14.26%), Carbendazim (14.57%), and Difenconazole (30.84%). Least effective fungicides recorded @ 1000 ppm Carbendazim (29.39%), mencozeb (47.44%), and propineb (53.3%). Least effective fungicides recorded @ 1500 ppm Carbendazim (67.02%), Difenconazole (72.98%).

The result of present studies are in agreement with the findings of viz., Arun Kumar (2008) [5] who reported that out of nine different fungicides tested in vitro, Propiconazole, Hexaconazole at all the concentration (0.1%, 0.2% and 0.3%) completely inhibited the mycelial growth of *A. alternata* infecting cotton. Thaware et al. (2010) [7] evaluated different fungicides under in vitro conditions against leaf blight of cotton caused by *A. alternata* and showed that Mancozeb @ 0.2 per cent and Propiconazole @ 0.05 per cent completely inhibited the mycelial growth of test fungus.

Berman et al. (2015) [8] estimated different fungicides against leaf blight of tomato caused by *A. alternata* @ 0.2 per cent concentration. They concluded that Bavistin showed 100 per cent inhibition followed by Captan (81.2%), Nystatin (73%).

Mohan et al. (2018) [9] evaluated eleven fungicides against leaf blight of cotton caused by *A. macrospora* under in vitro conditions. Among all the tested fungicides mancozeb, carbendazim, hexaconazole, propiconazole and carbendazim + mancozeb showed complete inhibition (100%) at all the tested dosages followed by captan + hexaconazole (89.74%), thiram and captan (78.89%) and azoxystrobin showed least inhibition (56.81%) compared to untreated control (Nene and Thapliyal, 1979) [10].

Raut et al. (2019) [11] evaluated seven non systemic fungicides viz., Chlorothalonil 75WP, Propineb 70WP, Mancozeb 75WP, Copper oxychloride 50WP, Captan 50WP, Thiram 75WP and

Ziram 27EC each @ 1500 and 2000 ppm concentration against *A. macrospora* which causes leaf blight of cotton. Among eleven fungicides maximum per cent inhibition was observed with Mancozeb (86.95%), followed by Thiram (84.68%), Ziram (81.76%), Propineb (80.23%) and Copper oxychloride (75.57%), whereas, it was comparatively minimum with Chlorothalonil (55.29%) and Captan (73.10%). The radial growth of Mancozeb and Copper oxychloride @ 1500 ppm observed was 14.50 and 12.50 mm respectively with per cent inhibition of 83.8 and 86.1 per cent respectively. While, the radial growth of Mancozeb and Copper oxychloride @ 2000 ppm observed was 12.5 mm and 6.46 mm respectively and showed 86.1 and 92.8 per cent inhibition respectively.

Among the different twelve fungicides tested, Tricyclazole, and Tebuconazole + Trifloxystrobin at 500, 1000 and 1500 ppm were found most effective against *Alternaria cucumeria* var. *cymopsidis*, with maximum growth inhibition (100%). Tricyclazole, and Tebuconazole + Trifloxystrobin were the best fungicides for control of *A. cucumeria*. It was concluded that among the all experimented fungicides in vitro, Tricyclazole, and Tebuconazole + Trifloxystrobin at their recommended doses as well as below and above their recommended doses showed complete inhibition of mycelial growth of *A. cucumeria*.

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