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Management of Leaf spot of Clusterbean caused by *Alternaria cucumerina var.cyamopsidis*

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Abstact: Cluster bean also called as Guar (C*yamopsis tetragonoloba* (Linn.) Taub) is an important vegetable crop grown across the country. Leaf spot caused by *Alternaria cucumerina var cyamopsidis* is the most devastating disease reported to cause major economic yield losses. Disease management is a challenging task as there is a continuous presence of the pathogen throughout the season many fold speculations on possible survival of the pathogen on same alternative host grown in and around garden. Hence, efforts were made to find out the effective management strategy involving host resistance, biological and chemical protection.

Clusterbean (C*yamopsis tetragonoloba* (Linn.) Taub.), commonly known as a gaur, derived from Sanskrit word "Gau Aahar" means cow fodder. Apart from being a good vegetable, it is a deep rooted summer annual crop, having considerable importance as an industrial crop of the arid zone in recent years. Center of origin of the crop is confined to Indian subcontinent. India is the largest producer of guar and contributes around 80 per cent of total guar production in the world. Cropping area is near of about 5.60 million ha with the production of 2.72 million tonnes. The average yield is around 485 kg/ ha. It is an important crop of north India, but appreciable area is also reported from south India.

The productivity of gaur has been low and static, mainly because of its cultivation in marginal and sub marginal lands under rainfed conditions. In Karnataka, the crop is cultivated in both *kharif* and *rabi* season, mostly as mixed and intercrop with inadequate adoption of plant protection measures. Among the different biotic constraints, diseases took the heavy toll in reducing the yield attributes. The crop is known to suffer from various diseases viz; *Alternaria* leaf spot, Powdery mildew, Anthracnose, Bacterial blight, *Cercospora* leaf spot and *Myrothecium* leaf spot. Among these diseases, leaf spot caused by *Alternaria cucumerina* var. *cyamopsidis* (Rangaswami and Rao) Simmons is most common and prevalent on cluster bean. The disease appears regularly in all the cultivated areas with varying degree of severity and reported to cause huge losses to the growers and emerged as a serious constraint for the production of cluserbean in terms of vegetable, grain and fodder production. This disease alone has been reported to cause reduction in yield by 55.76 to 58.70 per cent under artificial epiphytotic condition (Gupta, 1994). The disease appears regularly on the crop in a mild to severe form, since the pathogen is seed borne in nature (Sowell, 1965). Effective management of *Alternaria* leaf spot is a challenging task due to the continuous presence of pathogen throughout the season and many fold speculations on possible survival of the pathogen on some alternate hosts grown in and around the garden. As single method of disease control does not suits all the time. Hence,

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efforts were made to find out the effective management strategy involving biological and chemical protection along with host resistance.

MATERIAL AND METHODS

Assesment of bioefficacy of biocontrol agents against the pathogen by *in vitro*

The efficacy of bio control agents was determined by dual culture technique. The Fungal bio agents used in experiment were *Tricoderma viride, Tricoderma harzianum* and *Tricoderma virens.* Bacterial bio agents were *Pseudomonas fluorescens* and *Bacillus subtilis.* It was done by taking 20 ml of sterilized melted potato dextrose agar medium and plated on to the Petriplates and then allowed to solidify. A disc of 5 mm in diameter of each of actively growing three days old fungal bio control agent was cut with sterile cork borer and placed at one end of plate over the PDA medium. To test the efficacy of antagonistic bacterium, the culture was streaked at one end of the plate and on the opposite side of an antagonist; mycelial disc of the pathogen was placed. The petriplates inoculated with pathogen at one end alone served as a control. Then the inoculated plates were incubated at 27 ± 1°C for one week. The experiment was separately done for each of the biocontrol agent. Three replications were maintained for each treatment. The observations were recorded for growth of the pathogen both in control and treated plates. Per cent inhibition of the growth of the test pathogen was calculated using the formula given by (Vincent, 1947) as follows.

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

I = per cent inhibition

C= Germination of sporangia in control

T= Germination of sporangia in treatment

Efficacy of fungicides against the pathogen by *invitro*

In vitro assay of fungicides was carried out by using poison food technique (Zentmeyer, 1955) to assess the efficacy of fungicides in suppressing the growth of the pathogen. The systemic fungicides were evaluated at 0.05, 0.1 and 0.15 per cent concentration and non systemic fungicides at 0.1, 0.2 and 0.3 per cent concentrations. The list of fungicides evaluated is furnished below.

a) Non-systemic fungicides

Sl. No.	*Common name*	*Chemical name*
1	Copper oxy chloride	Copper oxy chloride
2	Chlorothalonil	2,4,5,6 tetrachloro isophthalo nitrate
3	Mancozeb	Manganese ethylene bis dithiocarbamate
4	SAFF [Carbendazim (12%) + Mancozeb (63 %)]	Methyl 1-1-2 benzimidozole carbamate + Manganese ethylene bis dithiocarbamate

b) Systemic fungicides

Sl. No.	*Common name*	*Chemical name*
1	Hexaconazole	2-(2,4-dichloro phenyl)-1-(1H-1, 2,4-triazol-1-yl) hexan-2-ol
2	Propiconazole	1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl] methyl]-1,2,4-triazole
3	Difenconazole	Cis, trans-3-chloro-4-(4-methyl-2-(1H-1,2,4-triazole-1- yl)-1,3-dioxalan-2yl) phenyl-4 chloro phenyl ether
4	Thiophanate methyl	1,2, bis (3-methoxy cabory l-2-thioureidobenzene)
5	Tebuconazole	1-(4-Chlorophenyl)-4,4-dimethyl-3-(1*H*, 1,2,4-triazol-1-ylmethyl)pentan- 3-ol
6	Carbendazim	2-methoxy-carbamoyl-benzimidazole

Based on the active ingredient, chemicals were weighed, mixed with molten potato dextrose agar to get the required concentrations and then cooled. Twenty ml of the poisoned medium was poured into the sterilized petri plates. Four replications were maintained for each treatment. The plates were then inoculated with mycelial disc of the fungus (5 mm in diameter) and inoculated plates were incubated at 27±1°C. Suitable control was maintained without adding fungicides. Observations were recorded for the growth of the pathogen in treated plates. The percent inhibition of growth in each treatment was calculated by using the formula (Vincent, 1947) as furnished in the previous experiment.

Field evaluation of fungicides

Field experiment was conducted during *kharif* 2016 under irrigated conditions to know the field efficacy of different fungicides. The experiment was laid out in randomized block design (RBD) with the plots size of 3mx3 m spaced at 45x15 cm with three replications. The first spray of each treatment was taken up as soon as the disease symptoms are noticed. A total of three sprays were given at an interval of 15 days. The disease intensity in both treated and control plot was recorded by using 0-5 scale. Per cent disease index was calculated and data was analyzed statistically.

Screening of varieties /genotypes

Thirteen different genotypes were screened for their response against the disease through artificial inoculation of pathogen under pot house condition. Plants of one month old were artificially inoculated with spore suspension of 10 spores/ml prepared in sterile distilled water from 15 days old culture. Observations for the development of symptoms were recorded after 30 days of inoculation. All the leaves in each plant were graded based on the severity of infestation by using 0-5 scale given by Mayee and Datar (1986) as furnished below. Further per cent disease index was calculated by using the formula given by Wheeler (1969).

Table 1: 0-5 scale given by Mayee and Datar (1986)

Disease score	*Detail of infection*	*Categories*
0	<1% leaf area infected	Immune
1	1-5% leaf area infected	Resistant
2	6-20% leaf area infected	Moderately resistant
3	21-40% leaf area infected	Moderately susceptible
4	41-70% leaf area infected	Susceptible
5	>71% leaf area infected	Highly susceptible

RESULTS AND DISCUSSION

***In-vitro* evaluation of bio-agents against the pathogen**

In order to explore the possible antagonistic potential, different strains of *Trichoderma viz.*, Tr-5, Tr-9 and Tr-12 along with strains of *Pseudomonas fluorescense* (Pf-1) and *Bacillus subtilis* (Bs-16) were subjected for their efficacy against the pathogen.Studies were conducted by employing dual culture technique under *in-vitro* condition as narrated in material and methods.

The data presented in the Table 2, clearly indicated that all the five bio-control agent under the study have significantly inhibited the growth of the pathogen with superior efficacy exhibited by Tv-9 strain (*Trichoderma viride*) of 73.82 per cent of mycelial inhibition. The next best bio-control agent found to be Tv-13 strain (*Trichoderma virens*) with 69.53 per cent inhibition, followed by Th-5 strain (*Trichoderma harzianum*) with 51.08 per cent inhibition. *Pseudomonas fluorescense* was found to be moderately effective with 36.33 per cent inhibition and *Bacillus subtilis* exhibited very least efficacy in arresting the growth of the pathogen by 26.15 per cent inhibition (Fig. 1).

In general, bio-agents are found to be effective, eco friendly and cost effective in controlling the diseases. Therefore the novel bio-control agent such as *Trichodermaviride* (Tv-5), *Trichoderma harzianum* (Th-12), *Trichoderma virens* (Tv-9), *Pseudomonas fluorescense* (*Pf-1*) *and Bacillus subtilis* (*Bs-16*) were evaluated *in-vitro* against *A. cucumerina* var. *cyamopsidis* through dual culture technique. Results showed that all the bio-agents significantly reduced the per cent growth inhibition of the test fungus. Among the bio-agents, *T. viride* was found superior over others in inhibiting the test fungi by 73.82 per cent. The next best antagonism was exhibited by *Trichoderma virens, which inhibited the test fungi to an extent of 69.53 per cent followed by Trichoderma harzianum* (51.08%). While the bacterial antagonist such as *Pseudomonas fluorescense was moderately effective in inhibiting and B. subtilis* was *least effective (*Fig. 2).

Among the bio-control agents tested, inhibition of radial growth was maximum in case of *Trichoderma viride* (56.63 %), followed by *Trichoderma harzianum* (46.83%). But *Bacillus subtilis* (28.47%) and *Pseudomonas fluorescens* (28.44%) were less effective in inhibiting the radial growth of the pathogen. (Neelakanth *et al.,* 2014). Rahman *et al.,* (2015), maximum inhibition of colony growth of *A. porri* was observed by *Trichoderma viride* followed by *Trichoderma harzianum*, *Trichoderma koningii* and

least effective was shown by *Bacillus sp*. Probable mechanism could be the higher competitive ability, stimulation, and antibiosis by *Trichoderma* isolate over the test pathogen.

Table 2: *In-vitro* evaluation of bio-agents on inhabiting of mycelial growth of *Alternaria cucumerina* var. *cyamopsidis*

Sl. No	*Bio-agents*	*% Inhibition of mycelialgrowth*
1	*Trichoderma viride* (Tv-9)	73.82* (59.23)**
2	*Trichoderma harzianum* (Th-5)	51.08 (45.62)
3	*Tricoderma virens* (Tv-13)	69.53 (56.50)
4	*Pseudomonas fluorescense* (Pf-1)	36.33 (37.07)
5	*Bacillus subtilis* (Bs-16)	26.15 (30.76)
6	Control	0.0
SEm ±		0.55
CD at (1 %)		2.53

Original values ** Arcsine values

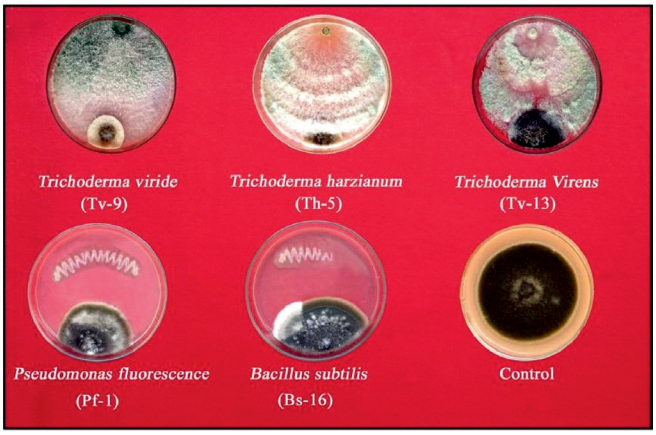

Figure 1: Effect of different bio control against the growth of *Alternaria cucumerina var. cyamopsidis invitro*

2. Evaluation of systemic and non systemic fungicides against the pathogen by *in-vitro*

The efficacy of six systemic and four non systemic fungicides was tested against the growth of *Alternaria cucumerina* var. *cyamopsidis*. The chemicals were evaluated at three different concentrations by "Poisoned food Technique". The results (Table 3 and fig.2) reveled that, among the systemic fungicides, hundred per cent inhibition of mycelial growth was recorded in the plates treated with *viz.,* difenconazole, propiconazole, tebuconazole and hexaconazole at all the three concentration. The fungicide thiophanate methyl exhibited the poor efficacy with 18.83 per cent of inhibition, while carbendazim was found least effective (14.76 %) in inhibiting the growth of the pathogen.

Between the concentrations of each systemic fungicide, efficacy was significant from lower to higher concentration with greater efficacy at higher concentrations for the chemicals like thiophanate methyl and carbendazim. Except these two chemicals, there was no significant difference between the concentrations on the efficacy of other systemicfungicides.

Interaction effect among the systemic fungicides and concentrations reveled that, the fungicides *viz.,* difenconazole, propiconazole, tebuconazole and hexaconazole were highly effective each at 0.05 per cent concentration with 100 per cent inhibition of mycelial growth. The efficacy between these fungicides was non-significant.

Among the four non systemic fungicides, carbendazim + mancozeb (SAFF) was found to be significantly superior in inhibiting the growth of the fungus, recording 88.59 per cent of mycelial inhibition, followed by mancozeb (79.14 per cent) and copper oxychloride (71.57 %). The other fungicide *viz.,* chlorothalonil was found least effective with an inhibition of 34.66 per cent. (Table 4 and fig. 3)

Between the concentrations of each non-systemic fungicides, efficacy was significant from lower to higher concentration with greater efficacy at higher concentrations.

Interaction effect among the non-systemic fungicides and concentrations indicated that, carbendazim + mancozeb (SAFF), mancozeb and copper oxychloride at 0.3 per cent concentrations were highly effective with mycelial inhibition of 100, 83.64 and 81.68 per cent respectively. The efficacy between the fungicides like mancozeb and copper oxychloride at 0.3 per cent concentration was found non-significant. carbendazim + mancozeb (SAFF) at 0.1 and 0.2 per cent concentration inhibited the growth to the extent of 81.56 and 84.21 per cent respectively and

Table 3: *In-viro* evaluation of systemic fungicides on inhibition of mycelial growth of Alternaria cucumerina var. cyamopsidis

Sl. No	*Fungicides*	*Inhibition of mycelial growth (%)*			
		Concentration (%)			*Mean*
		0.05	*0.1*	*0.15*	
1	Difenconazole	100* (90.00)**	100 (90.00)	100 (90.00)	100 (90.00)
2	Propiconazole	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
3	Tebuconazole	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
4	Hexaconazole	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
5	Thiophanate methyl	10.69 (18.99)	19.11 (25.92)	26.70 (31.07)	18.83 (25.72)
6	Carbendazim	9.09 (17.71)	15.70 (23.22)	19.51 (26.21)	14.76 (22.59)
7	Control	0.00	0.00	0.00	0.0

was found on par with the efficacy of mancozeb and copper oxychloride at 0.3 per cent. The fungicides like carbendazim + mancozeb (SAFF), mancozeb and copper oxychloride inhibited more than 50 per cent of radial growth even at 0.1 per cent concentration. Whereas, chlorothalonil found to be the least effective among the non-systemic chemicals with mycelial inhibition of 49.21 per cent at 0.3 per cent concentration.

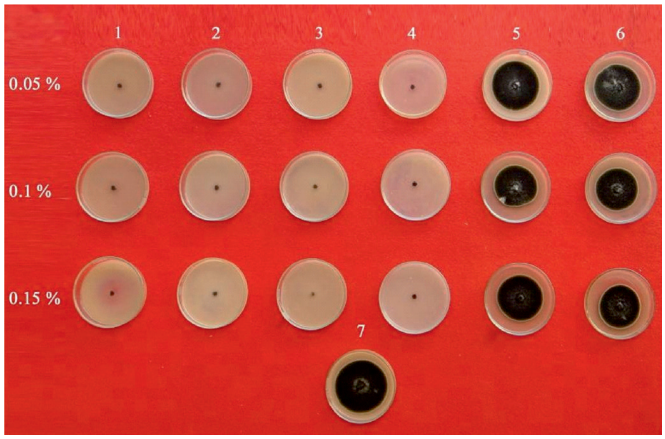

Figure 2: Effect of systemic fungicides on growth of *A. cucumerina* var. *cyamopsidi*

Table 4. *In-viro* evaluation of non systemic fungicides on inhibition of mycelialgrowth of *A. cucumerina* var. *cyamopsidis*

Sl. No	*Fungicides*	*Inhibition of mycelial growth (%)*			
		Concentration (%)			*Mean*
		0.1	*0.2*	*0.3*	
1	Carbendazim + mancozeb	81.56* (64.58)**	84.21 (66.59)	100 (90.00)	88.59 (70.26)
2	Mancozeb	69.11 (53.14)	75.68 (60.53)	83.64 (66.16)	76.14 (60.76)
3	Copperoxy chloride	64.00 (53.14)	69.03 (56.19)	81.68 (64.71)	71.57 (57.78)
4	Chlorothalonil	20.43 (26.86)	34.36 (35.89)	49.21 (44.55)	34.66 (36.07)
5	Control	0.0	0.0	0.0	0.0

	Fungicides (F)	*Concentration (C)*	*F × C*
SEm ±	0.57	0.49	0.98
CD at (1 %)	1.59	1.38	2.75
CV	2.63		

* Original values ** Arcsine value

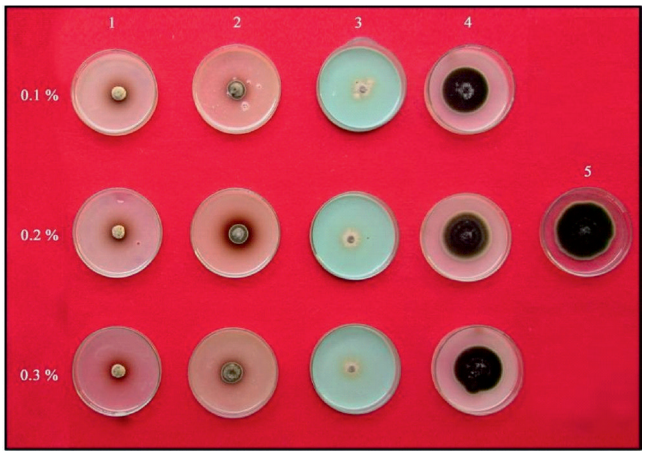

Figure 3: Effect of non systemic fungicides on growth of *A. cucumerina* var. *cyamopsidis*

Field evaluation of fungicides

A study was taken up during *Kharif* 2016 to assess the field efficacy of six systemic and four non systemic fungicides against the *Alternaria* leaf spot of clusterbean. The experiment was taken up at ARS Bheemarayanagudi.

Results (Table 5 and Fig 4) of field trails indicated that, among the fungicides evaluated, hexaconazole at 0.05 per cent was found significantly effective in controlling the disease by recording the least disease index of 22.46

PDI followed by propiconazole (23.46 PDI) and tebuconazole (24.26 PDI), which exhibited on par efficacy with each other. The other fungicides such as difenconazole (0.05 %), carbendazim + mancozeb (0.3 %) and mancozeb (0.3 %) were found moderate effective against the disease. The efficacy exhibited by the carbendazime was significantly least as compaired to all the fungicides under evaluation. The highest disease incidence of 51.53 PDI was recorded in untreated control plot.

All the fungicides tested by *in-vitro*, were further tested under field in natural condition for the management of blight of clusterbean. Among the systemic fungicide tested, hexaconazole, propiconazole and tebuconazole at 0.05 per cent concentration were found most effective in reducing the disease followed by difenconazole. Whereas among the non-systemic fungicides carbendazim + mancozeb and mancozeb at 0.3 per cent concentration ceases the disease to an extent of 28.86 and 29.66 PDI respectively as compared to untreated control (Fig 9). Akbari and Parakhia (2007) reported that, the field performance of propiconazole (0.05%) was superior in controling sesame blight caused by *A. alternata*. Hexaconazole (0.05%) was also found effective exhibiting the on par efficacy with propiconazole. Chattannavar *et al.*, (2004) observed that, the new chemical tebuconazole (folicure) at 0.05 and 0.07 per cent was very effective against *Alternaria* blight followed by copper oxychloride. Mesta (2006) reported that, hexaconazole (0.1%) and propiconazole (0.1%) were very effective in minimizing the *Alternaria* blight and resulted the higher yield of sunflower. Neema and Duhoon, (2007) reported that propiconazole and mancozeb were found effective against *A.* sesami.

Table 5: Field efficacy of different fungicide in controlling the *Alternaria* leaf spotof clusterbean

Sl. No	*Treatments*	*Conc (%)*	*Percent disease index(PDI)*	*Per cent reduction over control*
			3rd Spray (75 days)	
1	Hexaconazole	0.05	22.46* (28.28)**	56.41
2	Tebuconazole	0.05	24.26 (29.50)	52.92
3	Propiconazole	0.05	23.46 (28.95)	54.47
4	Thiophanate methyl	0.15	36.00 (36.87)	30.13
5	Carbendazim	0.15	36.53 (37.19)	29.10
6	Difenconazole	0.05	26.93 (31.26)	47.73
7	Carbendazim + mancozeb	0.3	28.86 (32.50)	43.99
8	Chlorothalonil	0.3	33.46 (35.34)	35.06
9	Mancozeb	0.3	29.66 (33.00)	42.44
10	Copper oxychloride	0.3	31.20 (33.95)	39.45
11	Control	---	51.53 (45.88)	---
	SEm±	---	0.57	---
	C.D. at 5%	---	1.68	---

Original values ** Arcsine values

Field view of experiment

Hexaconazole (5 % SC) treated plot Untreated check plot

Figure 4: Field efficacy of different fungicides against *Alternaria* leaf spot of clusterbean

Varietal screening

Cultivation of resistant or tolerant variety against any disease is economical, ecofriendly and cost effective method of disease management. Hence the present study was aimed at identification

of resistant sources to *Alternaria* leaf spot of clusterbean. Among thirteen genotypes of clusterbean comprising both gum gaur and vegetable types were obtained from ARS Bheemarayanagudi. The genotypes were planted in pots during *Kharif* 2016 and screened for disease resistance.

Among thirteen genotypes screened, GAUG-13 and GG-1 were found resistant. Genotypes like HG-365, HG-563, RGC-936, Gourishankar-9 and Amul-51 were moderately resistant. Selection-51, Deshi and Raichur local indicated moderately susceptible category.. Further, the genotypes like RGC-1017, Amrit-11 and PNB were found susceptible to the disease. (Table 6 and Fig 5, 6).

Host plant resistance is one of the chief and economic approach for the management of crop diseases. Use of resistant varieties is the simplest way to manage the disease by resource poor farmers. Management through resistant varieties is permanent, cost effective and eco friendly as compare to other practices of management. In the present study, thirteen cultivars of clusterbean were screened under pot house condition to identify the resistant sources against the *Alternaria* leaf blight disease.

The results showed that, out of thirteen cultivars tested, two cultivars GAUG-13 and GG-1 were found resistant. The genotypes *viz.*, HG-365, HG-563, RGC-936, Gourishankar-9 and Amul-51 were moderately resistant. Selection-51, Deshi and Raichur local were moderately susceptible, the cultivars like RGC-1017, Amrit-11 and PNB found to be susceptible.

Yenjerappa (1989) reported that, out of eighteen different genotypes of clusterbean screened against the disease, only two genotypes GG-3 and HFG-408 were found resistant, the genotypes Dharwad local and RGC-471 were susceptible.

Saharan and Saharan, (2003) stated that, out of 186 genotypes of clusterbean evaluated, none were found free from the disease. However the genotypes like GAUG- 9008, GAUG-9407 and RGC-1014 were found resistant and the genotype PNB was highly susceptible.

Table 6. Screening of promising genotypes of clusterbean against *Alternaria* leaf spot

Sl. No	*Genotypes*	*Grade*	*Reaction group*
1	HG-365	2	MR
2	HG-563	2	MR
3	RGC-936	2	MR
4	RGC-1017	4	S
5	GAUG-13	1	R
6	Gourishankar-9	2	MR
7	GG-1	1	R
8	PNB	4	S
9	Amrit-11	4	S
10	Amul-51	2	MR
11	Selection-51	3	MS
12	Deshi	3	MS
13	Raichur local	3	MS

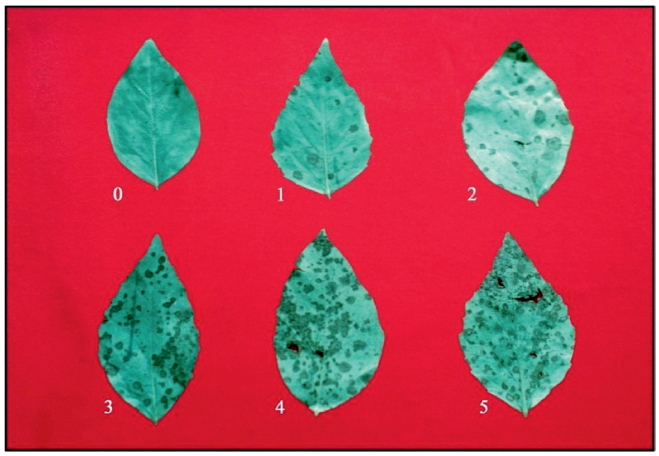

Figure 5: Disease scale used for screening genotypes of clusterbean against *Alternaria* leaf spot

Figure 6: Screening of different clusterbean genotypes against Alternaria leaf spot caused by *A. cucumerina* var. *cyamopsidis* under green house condition

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