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In vitro and *in vivo* field efficacy of different fungicides against *Alternaria brassicae* (Berk.) sacc. causing *Alternaria* leaf spot of cauliflower

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Abstract
The *Alternaria* leaf spot caused by *A. brassicae* (Berk.) Sacc is one of the destructive disease of Cauliflower in Konkan region of Maharashtra. In the present study, bio efficacy of seven fungicides tested under *in vitro* conditions against the pathogen. Among that, Mancozeb 75% WP (0.25 %) was completely inhibited the growth of the test fungus. It was followed by Propiconazole 25% EC (0.1%) which showed 96.29 per cent inhibition of the test fungus and was at par with Mancozeb. No sporulation was observed in Mancozeb 75% WP, Propiconazole 25% EC and Difenconazole 25% EC. Among the different fungicides tested under field conditions, all the fungicidal treatments were significantly superior over control after last spray. The treatment Mancozeb 75% WP was the most effective as it recorded minimum disease incidence (28 %). It was followed by Azoxystrobin 23% EC which recorded 29 % disease incidence.

Keywords: Cauliflower, *Alternaria brassicae* (Berk.) Sacc. Fungicides, Management, Sporulation and Mycelial growth

Introduction
Several factors are responsible for low production of cauliflower crop, among which diseases also play an important role. The important diseases of Cauliflower crop are leaf spot, Downy mildew, Damping off, Club root, Powdery mildew, White rust, Black rot, Bacterial soft rot and Cauliflower mosaic. Among these diseases *Alternaria* leaf spot is a serious disease of cauliflower. About 20 to 80 per cent loss in yield and 59 per cent loss in seed may occur due to this disease. The disease appears as minute specks on the leaves, which enlarge over a time and result in substantial lesions with concentric rings where spores are produced. The disease starts from lower leaves and slowly progresses towards the upper shoots, leaves, petioles, pods and heads. Defoliation of the outer leaves may occur on severely infected plants and extensive trimming may be required to remove infected leaves from the cabbage head at harvest. In susceptible varieties, apart from yield, significant reduction in quality may occur.
During recent years the fungal diseases were found to occur in severe proportions under favorable environmental conditions particularly during *Rabi* season. *Alternaria* leaf spot caused by *Alternaria brassicae* has been reported to inflict heavy yield losses (Pattanamahakul and Strange, 1999; Azevedo *et al.*, 2000; Peruch *et al*., 2006; Chauhan *et al*., 2009; Mishra *et al*., 2009; Deep and Sharma, 2012 and Sharma *et al*., 2013) [14, 1, 15, 2, 11, 4, 17].
Alternaria leaf spot is difficult to control because the fungus penetrates in infected seed, plant debris in soil and collateral hosts such as other cruciferous crops in the vicinity of the main crop or weeds. One of the most effective and old method for disease control is the use of fungicides. There are several fungicides which are being commercially available while several others are being evaluated in different laboratories. Even though cauliflower is not a regularly cultivated crop in Konkan region, some progressive vegetable growers are switching to this crop due to its high marketability. Considering importance of the crop and disease, present study on *Alternaria* leaf spot of cauliflower was planned and conducted to evolve *in vitro* and *in vivo* management strategy.

Materials and Methods
In vitro evaluation of fungicides
The laboratory experiments in the present study were conducted in the Department of Plant Pathology, Dr. DBSKKV, Dapoli during 2015-16. The principle involved in poisoned food technique is to poison the nutrient medium with a fungi-toxicant and then allowing the test fungus to grow on it and finally recording the extent of growth (Nene and Thapliyal, 1997) [13]. The details of treatments used are mentioned below.

Potato dextrose agar medium (PDA) was used as basal medium and dispensed in 100 ml aliquots in each 250 ml Erlenmeyer conical flasks, which were sterilized at 1.054 kg/cm^2 pressure for 20 minutes. The quantity of every fungicide for each concentration was calculated for 100 ml medium separately. The weighed quantity of each fungicide was added in lukewarm PDA at 40 ± 2°C, mixed thoroughly and poured into sterilized Petri plates and allowed to solidify. The mycelial discs of 5 mm diameter were cut from 7 day old culture of the test fungus with the help of a sterilized cork borer. A single disc was transferred aseptically to the centre of each plate already poured with poisoned medium. The plates with PDA without fungicide but inoculated with fungal culture, served as control.

List of fungicides tested under *in vitro* and *in vivo* against the pathogen.

T. No.	Name of the fungicide and formulation	Trade name	Conc. (%) tested
T_1	Azoxystrobin 23% EC	Amistar	0.1
T_2	Carbendazim 50% WP	Fungiguard	0.1
T_3	Copper hydroxide 77% WP	Kocide 101	0.1
T_4	Mancozeb 75% WP	Dithane M-45	0.25
T_5	Difenconazole 25% EC	Score	0.1
T_6	Thiophanate methyl 70% WP	Roko	0.1
T_7	Propiconazole 25% EC	Tilt	0.1
T_8	Control	-	-

The plates were incubated at room temperature (27 ± 2°C). Three replications of each treatment were maintained. The observation on colony diameter of the fungus were recorded when Petri plate in control treatment was fully covered with mycelial growth. Per cent inhibition of growth of the test pathogen was calculated.

Per cent inhibition of growth of the test fungus was calculated by following formula (Horsfall, 1956) [7].

$$X = \frac{Y - Z}{Y} \times 100$$

Where, X = Per cent inhibition, Y = Growth of fungus in control (mm), Z = Growth of fungus in treatment (mm)

Field evaluation of fungicides

The field experiment was conducted on variety "Super-fast crop" of cauliflower during *Rabi* 2015-16 at the Botany farm, College of Agriculture, Dapoli.

Raising of cauliflower seedlings

The seeds of cauliflower were sown on in portrays containing cocopeat as growing medium. The trays were staked one above the other and the uppermost tray was covered with a clean plastic sheet for 4 days. Seeds germinated within 4 days and then each tray was arranged separately on raised beds in shed net. The trays were watered regularly to maintain sufficient moisture. The Cauliflower seedlings were ready for transplanting in 25 days.

Preparation of experimental plot

The experimental plot was prepared with a tractor drawn mould board plough by two crisscross ploughings with tractor operated cultivator. Planking was done to level the field. The experiment was laid in randomized block design. Three replications were maintained per treatment.

Transplanting of seedlings

Initially light irrigation was given to the experimental plot in the morning so as to achieve optimum moisture in the soil to facilitate establishment of seedling during transplanting. On the same day evening, 25 days old healthy seedling were carefully uprooted and immediately transplanted in the field. Light irrigation was given to the plot immediately after transplanting for better crop stand.

Schedule of spraying of Fungicide:

The crop was observed carefully for initiation of the disease. First spray of each fungicide treatment was given immediately after disease incidence was noticed. Two more sprays of fungicides were given at an interval of 15 days.

Method of recording observations:

Five plants per treatment per replication were randomly selected for recording disease incidence of *Alternaria* leaf spot. Initial observations were recorded before first spray and final observations were recorded 15 days after the last spray on the basis of development of spots and lesions by considering the whole leaf area as hundred per cent.

Observations on disease incidence:

The disease intensity was recorded in 0-5 scale (Conn. *et al.* 1990) as described below.

Score/grade	Description
0	No disease
1	<5% leaf area affected
2	6-10% leaf area affected
3	11-25% leaf area affected
4	26-50% leaf area affected
5	>50% leaf area affected

Per cent disease intensity (PDI):

Per cent disease intensity was calculated by the following formula given by (McKinney, (1923)

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves examined x Maximum rating}} \times 100$$

Per cent disease control (PDC)

The per cent disease control was calculated by using the formula given below:

$$PDC = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

Statistical analysis

The data obtained were statistically analysed by the methods suggested by Gomez and Gomez (1986) [6]. The standard error and critical difference were worked out and the results obtained were compared statistically.

Results and Discussion

***In vitro* evaluation of fungicides**

Seven fungicides belonging to different groups were tested for their efficacy against *A. brassicae* by employing poisoned food technique. The data obtained on the effect of different fungicides on the vegetative growth and sporulations of A. brassicae *in vitro* are presented in Table 1, PLATE-I and depicted in Fig-1.

The data presented in Table 1 revealed that, among the different fungicides tested under *in vitro* Mancozeb 75% WP (0.25 %) completely inhibited the growth of the test fungus. It was followed by Propiconazole 25% EC (0.1%) which showed 96.29 per cent inhibition of the test fungus and was at par with Mancozeb. The next fungicide in order of merit was Difenconazole 25% EC (0.1 %) which recorded 87.77 per cent inhibition. Copper hydroxide 77% WP (0.1 %) and Azoxystrobin 23% EC (0.1 %) showed 52.96 per cent and 49.25 per cent inhibition of *A. brassicae*, respectively over control and were at par with each other. In the treatment of Thiophanate methyl 70% WP (0.1%), 38.88 per cent inhibition of the fungus was achieved. Carbendazim 50% WP (0.1) was appeared as least effective fungicide which showed only 27.77 % inhibition of the test pathogen.

Table 1: *In vitro* efficacy of fungicides on growth and sporulation of *A. brassicae* (Berk.) Sacc.

T. No.	Name of the fungicide	Conc. (%)	Mean colony diameter (mm)	Per cent inhibition over control	Sporulation
T_1	Copper hydroxide 77% WP	0.1	42.33	52.96	+
T_2	Azoxystrobin 23% EC	0.1	45.66	49.25	+
T_3	Difenconazole 25% EC	0.1	11.00	87.77	-
T_4	Propiconazole 25% EC	0.1	3.33	96.29	-
T_5	Mancozeb 75% WP	0.25	0.00	100	-
T_6	Thiophanate methyl 70% WP	0.1	55	38.88	++
T_7	Carbendazim 50% WP	0.1	86.66	3.70	+++
T_8	Control		90.00	-	++++
S.Em.±			0.91		
C.D.at 1%			3.85		

Sporulation

- = No sporulation, +++ = Good,
+ = Poor, ++++ = Excellent.
++ = Fair,

No sporulation was observed in Mancozeb 75% WP (0.25%), Propiconazole 25% EC (0.1%) and Difenconazole 25% EC (0.1%). Poor sporulation was recorded in Copper hydroxide 77% WP (0.1%) and Azoxystrobin 23% EC (0.1%). Fair sporulation was observed in Thiophanate methyl 70% WP (0.1%). Good sporulation was observed in Carbendazim 50% WP (0.1%) as compared to control which showed excellent sporulation. The result findings are in agreement with those reported by Kumar *et al.*, (1999) [9] who reported that Carbendazim 0.05 and 0.1 per cent completely inhibited mycelial growth of *A. brassicae.* Beatrice *et al.*, (2004) showed that difenconazole was most effective against *A. brassicae and A. brassicicola*. Hossain and Mian (2004) reported that Mancozeb, Carbendazim and Propiconazole inhibited the mycelial growth of the *A. brassicicola* infecting cabbage. Surviliene *et al.*, (2006) reported that Azoxystrobin showed significant inhibitory effect on mycelial growth of the *Alternaria* spp. Mishra *et al.*, (2009) [11] reported that Propiconazole was the most effective against *A. brassicae* which showed maximum per cent inhibition of mycelial growth and was followed by Mancozeb and Azoxystrobin. Gaikwad (2013) also reported that Propiconazole, Difenconazole and Mancozeb at different concentrations completely inhibited the mycelial growth of *A. brassicae.*

Plate 1: Efficacy of fungicides on growth and sporulation of *Alternaria brassicae*

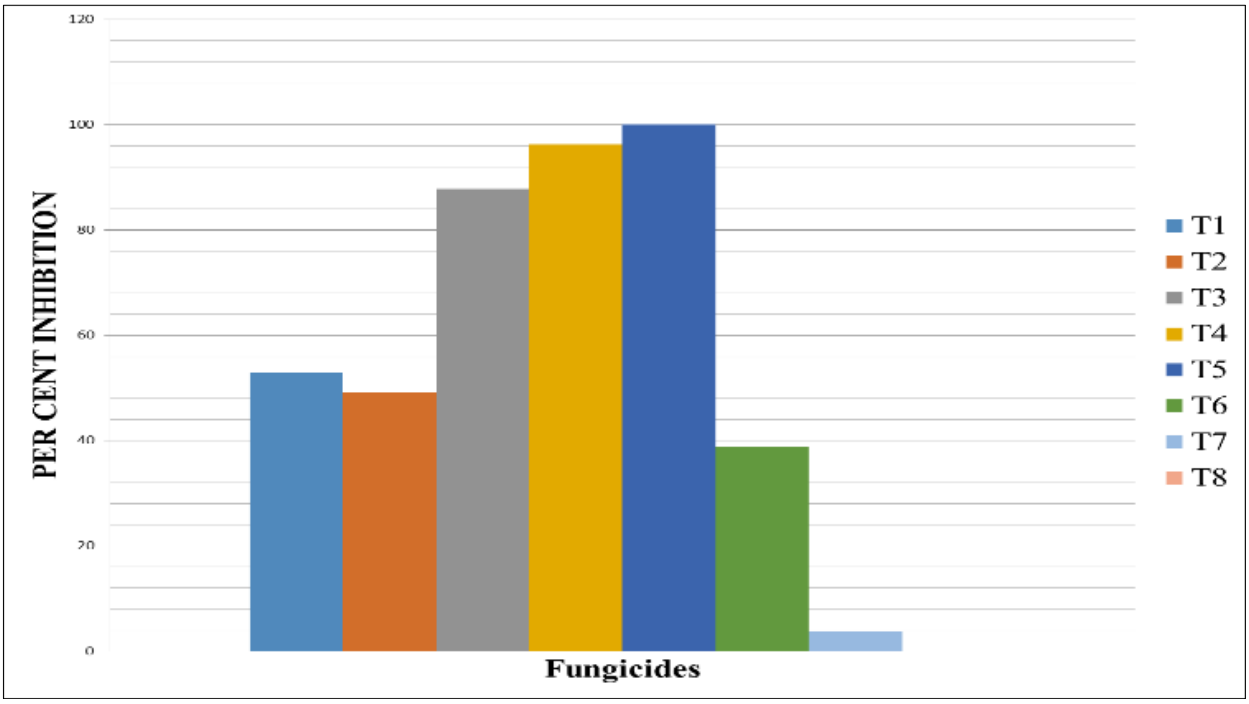

Fig 1: Efficacy of fungicides on growth and sporulation of A. brassicae (Berk.) Sacc.

Management of the disease in field conditions

The results of the experiment on evaluation of the fungicides against the disease under field conditions are presented in Table 2 and depicted in Fig-2.

Table 2: Per cent disease incidence (PDI) and per cent disease control (PDC) against *A. brassicae* (Berk.) Sacc.

T. No.	Treatments	Conc. (%)	Mean PDI before first spray	Mean PDI after last spray	PDC
T_1	Copper hydroxide 77% WP	0.1	19.60 (26.25)*	36.83 (37.36)	42.45
T_2	Azoxystrobin 23% EC	0.1	19.67 (26.32)	29.00 (32.58)	54.69
T_3	Difenconazole 25% EC	0.1	20.17 (26.61)	33.50 (35.35)	47.66
T_4	Propiconazole 25% EC	0.1	19.83 (26.41)	31.67 (34.24)	50.52
T_5	Mancozeb 75% WP	0.25	19.75 (26.38)	28.00 (31.93)	56.25
T_6	Thiophanate methyl 70% WP	0.1	19.83 (26.45)	36.00 (36.86)	43.75
T_7	Carbendazim 50% WP	0.1	20.00 (26.56)	34.00 (35.67)	46.88
T_8	Control		20.15 (26.67)	64.00 (53.13)	
S. Em.±			0.54	0.63	
C.D.at 5%			1.64	1.91	

(*Figures in parentheses indicate arc sin values)

It is apparent from the data presented in Table 2 that, among the different fungicides tested under field conditions all the fungicidal treatments were significantly superior over control after last spray. The treatment T_5 (Mancozeb 75% WP) was the most effective as it recorded minimum disease incidence (28 %). It was followed by T_2 (Azoxystrobin 23% EC) which recorded 29.00 per cent disease incidence. The treatment T_4 (Propiconazole 25% EC) was at par with T_3 (Difenconazole 25% EC). Treatment T_7 (Carbendazim 50% WP) showed 34 % disease incidence and it was followed by T_6 (Thiophanate methyl 70% WP) and T_1 (Copper hydroxide 77% WP) with 36 and 36.63 per cent disease incidence respectively over control (64 %). Maximum per cent disease control was achieved in treatment T_5 (Mancozeb 75% WP) and T_2 (Azoxystrobin 23% EC) which showed 56.25 per cent and 54.69 per cent disease control over control, respectively. Both the treatments were found statistically at par with each other. The next fungicides in merit of order were treatment T_4 (Propiconazole 25% EC) & T_3 (Difenconazole 25% EC) which recorded 50.52 per cent and 47.66 per cent disease control, respectively.

These were followed by treatment T_7 (Carbendazim 50% WP) with 46.88 per cent disease control and was at per with T_4. Treatment T_6 (Thiophanate methyl 70% WP) and T_1(Copper hydroxide 77% WP) showed 43.75 and 42.45 per cent disease control over control treatment, respectively and were statistically at par with each other. Sreedhar *et al.*, (2003) [18], showed that Azoxystrobin was the most effective fungicides as it reduced disease incidence to the tune of 88 to 93 per cent. Narain *et al.*, (2006) [12] reported that Mancozeb was the most effective fungicide against *A. brassicae*. Prasad and Lallu (2006) reported that first spray of Carbendazim (0.1%) + Mancozeb (0.2%) followed by two sprays of Mancozeb (0.2%) at early date of sowing (October) was the best combination in reducing the disease severity on leaves. Gaikwad (2013) showed that Mancozeb showed the least diseases incidence (15.30%) followed by Propiconazole (18.36%) and Copper oxychloride (20.65%).

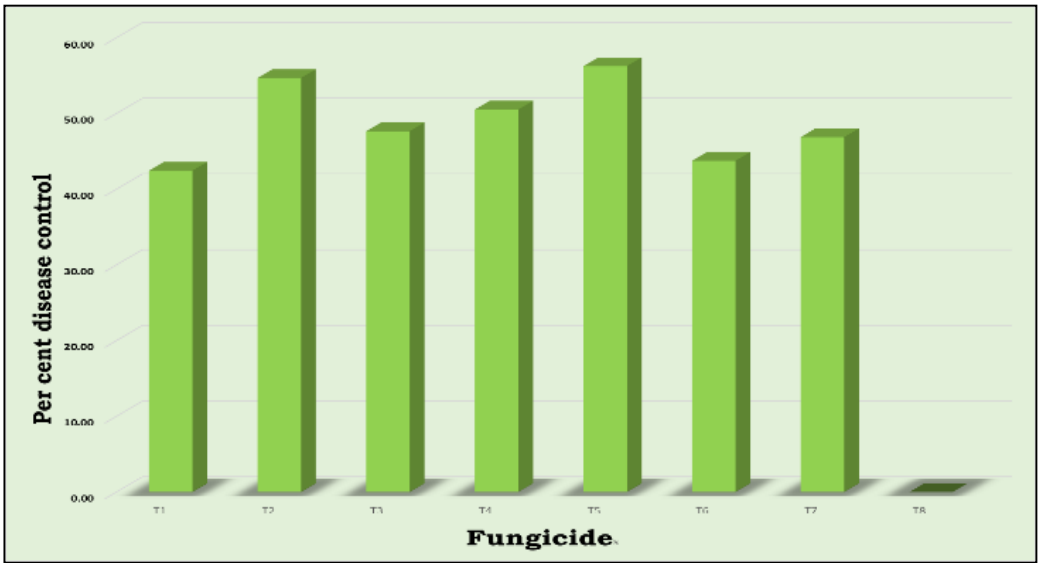

Fig 2: Management of the disease in the field by using different fungicides.

Conclusion

On the basis of the results of present study it can be concluded that *Alternaria* leaf spot of cauliflower caused by *Alternaria brassicae* (Berk.) Sacc. is an important disease of cauliflower in Konkan region. The fungicides *viz*., Mancozeb (0.25%) and Azoxystrobin (0.1%) are very effective against the pathogen under field conditions. Three sprays of Mancozeb (0.25%) or Azoxystrobin (0.1%) at an interval of 15 days will be the best strategy for management of *Alternaria* leaf spot of cauliflower. However, these findings need to be confirmed at different locations.

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