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Management of Leaf Spot and Flower Blight (*Alternaria tagetica*) Disease of Marigold

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ABSTRACT: The fungal pathogen *Alternaria tagetica* responsible for Alternaria leaf spot and flower blight, poses a significant threat to marigold production, potentially leading to a substantial 50-60 per cent decrease in yield. This study investigated the efficacy of various fungicides and botanicals against *A. tagetica*. *In vitro* bioassays were conducted for four fungicides—Azoxystrobin, carbendazim, mancozeb, and copper oxychloride—utilizing different concentrations: 25, 50, 75 ppm for systemic fungicides and 250, 500, 750 ppm for non-systemic fungicides. Additionally, four botanicals—Neem (*Azadirachta indica*), Darek (*Azadirachta melia*), Bhang (*Cannabis sativa*), and Datura (*Datura metel*)—were tested at concentrations of 10, 20 and 30 per cent. Remarkably, all fungicides and botanicals exhibited significant inhibition of mycelial growth even at the lowest concentrations. In the category of systemic fungicides, Azoxystrobin stood out at 75 ppm resulting in a remarkable 59.81 per cent mycelial inhibition. Among non-systemic fungicides, Mancozeb displayed superior efficacy at 750 ppm achieving a notable 73.89 per cent mycelial inhibition over the control. Neem, among the botanicals tested, proved most effective at 30 per cent concentration, inducing a substantial 81.56 per cent mycelial inhibition. Field trials further validated the effectiveness of these agents in managing Alternaria leaf spot and flower blight disease. Azoxystrobin, at 0.1 per cent concentration, emerged as the most effective, followed closely by Mancozeb resulting in 64.93 and 53.25 per cent disease reduction, respectively, surpassing other treatments.

Keywords: Leaf spot, systemic, non-systemic, fungicides, botanicals.

INTRODUCTION

Marigold, a prospective commercial flower, is gaining appeal due to its simple cultivation, high adaptability, and rising demand in the Indian subcontinent (Asif 2008). The flower represents purity and beauty. The wide spectrum of attractive colour, shape, size and good keeping quality attracts the attention of flower growers. It is widely cultivated and can be grown in most kinds of soils under sunny locations (Jarwar *et al*., 2021). Numerous pathogenic microbes, including bacteria, viruses, nematodes, and fungi, harm marigold plants and induce diseases that reduce production. In India, marigolds are frequently afflicted by leaf spot, foliage leaf blight, grey mould, powdery mildew, and anthracnose (Dhiman and Arora 1990). The most severe and widespread disease in the nation is leaf spot and flower blight (*Alternaria tagetica*) (Cotty and Mishaghi 1985). On flowers, stems, and foliage, the illness was visible. The disease progresses as a round to rectangular brownish spot that eventually turns dark brown to blackish, enlarges and coalesces to cover nearly the entire leaf and several branches, giving the plant a burned appearance. When the plant blooms, the inflorescence axis and flower heads are badly injured, along with the entire foliage, which leads to poor vegetative growth. In India, the disease is thought to have caused a loss of floral production of up to 55–60 per cent (Cotty and Mishaghi 1985). So, keeping this in view, the present study was carried out using different fungicides and botanicals against *A. tagetica* under *in vitro* as well as *in vivo* conditions.

MATERIALS AND METHODS

Isolation and purification of pathogen. During field surveys, disease samples showing characteristic symptoms *viz*., circular to oblong brownish spot, later these spots turned dark brown to blackish, enlarge coalesce to cover almost the entire leaves and part of branches giving the plant a burnt-up appearance and blighted appearance on the flowers were collected, bagged separately in perforated polythene bags, and brought to the laboratory for isolation of the associated pathogen with the diseased plants. Small bits from the diseased plant material were cut from the junction of diseased and healthy portion. The bits were then dipped in mercuric chloride (0.1%) solution for 30 seconds for surface sterilization and washed in three changes of

distilled water. The bits are then dried on sterilized filter paper and transferred to PDA plates under aseptic conditions of laminar air flow. The inoculated plates were incubated at 25±1°C in an incubator for 7- 10 days to allow the fungal pathogens to grow. The fungus was purified by hyphal tip method from a vigorously growing fungal culture. The visual and microscopic morphological features of the fungus were studied and recorded for comparison with the standard literature for confirmation of the identity of the fungus. The isolated pathogen was sub-cultured on PDA at a regular interval and preserved for further use in various experimental investigations.

Maintenance of pure culture:

Pure culture was maintained on potato dextrose agar (PDA) at 25±1°C after every 10-15 days for further use.

***In vitro* assay with fungicides and botanicals**

Systemic fungicides *viz*., Azoxystrobin and Carbendazim were evaluated at three concentrations (25, 50, 75 ppm) whereas non-systemic fungicides *viz.,* Mancozeb and Copper oxychloride were evaluated at 250, 500, 750 ppm and four botanicals *viz*., Neem, Darek, Bhang, Datura were evaluated at three concentrations (10, 20 and 30 %) against *Alternaria tagetica* using poisoned food technique (Sharvelle 1961). PDA without fungicide served as control. Each plate was inoculated with a 4 mm mycelial disc of the pathogen taken from seven-day old culture raised on PDA. The inoculated plates were incubated at 25±1°C till the fungus covered the plate in control. The growth of mycelium was recorded in each treatment and per cent inhibition over check was calculated using the following formula given by Vincent (1947):

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

where, I = per cent inhibition

C = Colony diameter in control (mm)

T = Colony diameter in fungicide amended medium (mm)

In vivo* evaluation of fungicides and botanicals against *Alternaria tagetica. The field experiment was conducted at Experimental farm with cultivar Pusa Basanti in Randomized block design (RBD) with 9 treatments and 3 replications including untreated control.

(Table 2) Four fungicides and four botanicals *viz.* Azoxystrobin (0.1%), Carbendazim (0.1%), Mancozeb (0.2%), copper oxychloride (0.3%) and Neem (*Azadirachta indica),* Darek (*Azadirachta melia*), Bhang (*Cannabis sativa*), Datura (*Datura metel*) at 30 per cent, respectively were sprayed two times. The first spray was given after the appearance of disease in experimental plot. Second spray of each treatment was given at 15 days intervals. In case of control, only water was sprayed. The observations on disease severity were recorded at 15 days after each spray. Percent disease severity was calculated by using 0-5 scale given by Hotchkiss and Baxter (1983) (Table 1).

Table 1: Disease Scoring Scale (Hotchkiss and Baxter 1983).

Disease rating	Host response	Symptoms/ Disease severity
0	HR	No symptoms on leaf
1	R	Spots covering 1-10% of the leaf area
2	MR	Spots covering 11-20% of the leaf area
3	MS	Spots covering 21-30% of the leaf area
4	S	Spots covering 31-40% of the leaf area
5	HS	Spots covering >40% of the leaf area

Table 2: Treatments for fungicide and botanical evaluation under field conditions.

Sr. No.	Treatments	Concentration (%)
T1	Azoxystrobin	0.10
T2	Carbendazim	0.10
T3	Mancozeb	0.20
T4	Copper oxychloride	0.30
T5	Neem	30
T6	Darek	30
T7	Bhang	30
T8	Datura	30

Statistical analysis: The statistical analysis of the collected data was done and data was analyzed by using OP STAT software.

RESULTS AND DISCUSSION

In-vitro* evaluation of fungicides against *A. tagetica. The bio-assay of four fungicides was conducted *in vitro* by using different concentration i.e., (25, 50, 75 ppm for systemic fungicides and 250, 500, 750 ppm for non-systemic fungicides) against *A. tagetica* by using poisoned food technique. Mycelial growth was measured after seven days of incubation. Mycelial growth of the test fungus, obtained in different concentrations of fungicides (Table 3) revealed that all the fungicides significantly inhibited the mycelial growth of the test pathogen at all concentrations. All the fungicides effectively controlled the fungal growth even at the lowest concentration. Azoxystrobin was the most effective and showed least fungal growth (36.17 mm) at 75 ppm (Plate 1). However, with the increase in concentration, the efficacy of all the fungicides enhanced and consequently the rate of fungal growth declined. Among other non-systemic fungicides, mancozeb was highly effective and recorded minimum fungal growth 25.50, 24.50, 23.50 mm at 250, 500 and 750 ppm, followed by copper oxychloride, while carbendazim was least effective recording the maximum fungal growth 46.27, 41.50, 39.50 per cent at 25, 50 and 75 ppm.

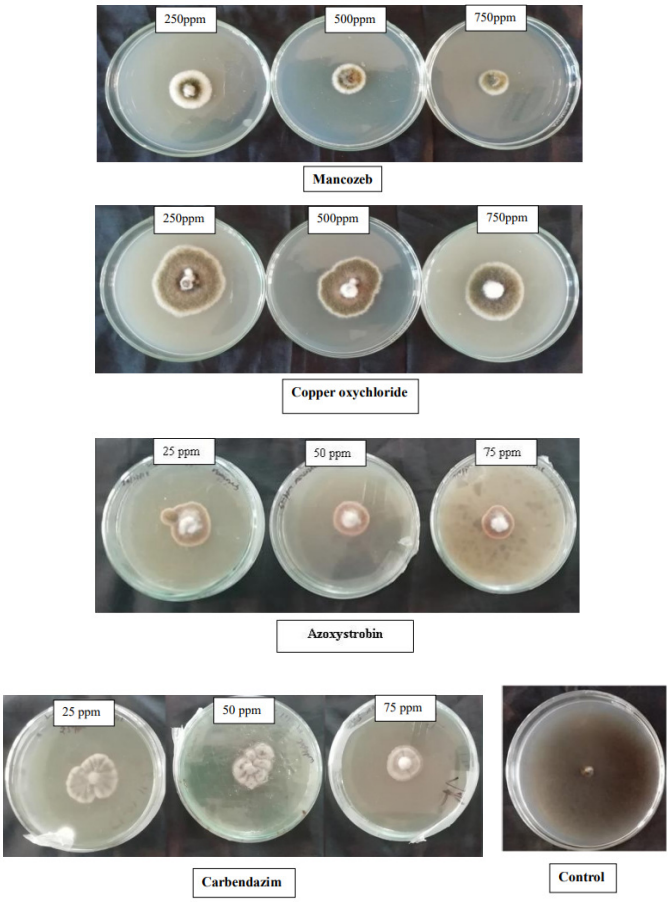

Plate 1. *In vitro* evaluation of fungicides against *A. tagetica*

Table 3: *In vitro* evaluation of fungicides against *Alternaria tagetica.*

Fungicide	Mycelial growth inhibition (%) at different Concentrations		
	250 ppm	**500 ppm**	**750 ppm**
Mancozeb	71.67 (57.84)	72.78 (58.55)	73.89 (59.27)
Copper Oxychloride	63.89 (53.06)	68.59 (55.92)	69.59 (56.54)
	25 ppm	**50 ppm**	**75 ppm**
Azoxystrobin	53.52 (47.02)	56.93 (48.98)	59.81 (50.66)
Carbendazim	48.59 (44.19)	53.89 (47.23)	56.11 (48.51)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	C.D. $_{p\geq0.05}$	S.E.(d)	
Fungicides (A)	0.92	0.31	
Concentration (B)	0.80	0.27	
Interaction	1.59	0.54	

**Values in the parentheses are angular transformed*

When the rate of inhibition of fungal growth was compared over control, the maximum inhibition was observed in Azoxystrobin (53.52, 56.92, 59.81%) whereas least in Carbendazim (48.60, 53.89, 56.11 %) at 25, 50 and 75ppm, respectively for systemic fungicides. On the other hand, the maximum inhibition was observed in Mancozeb (71.67, 72.78, 73.88%) whereas least in copper oxychloride (63.89, 68.59, 69.58 %) at 250, 500 and 750 ppm respectively for non-systemic fungicides. Thus, it was observed during the study that all the fungicides inhibited the mycelial growth of the fungus under *in vitro* conditions. Macdonald *et al*. (2007) reported that Azoxystrobin was linked to significantly higher total tuber yield for Russet Burbank in 2003 and Shepody in 2004 when compared to plots receiving no strobilurin fungicides. Mancozeb again reported effective at 750 ppm by giving 89.98 per

cent radial mycelial growth inhibition in case of *Alternaria solani* of tomato (Mate *et al*., 2005).

***In-vitro* evaluation of leaf extracts of botanicals against *A. tagetica*.** The bio-assay of four botanicals was conducted under *in vitro* conditions by using various concentrations (10, 20, 30%) against *A. tagetica* by poisoned food technique. Mycelial growth was measured after seven days of incubation. Mycelial growth of the test fungus, obtained in Table 4 at different concentrations of botanicals (Plate 2) revealed that all the botanicals significantly inhibited the mycelial growth of the test pathogen at all concentrations. All the botanicals effectively controlled the fungal growth even at the lowest concentration.

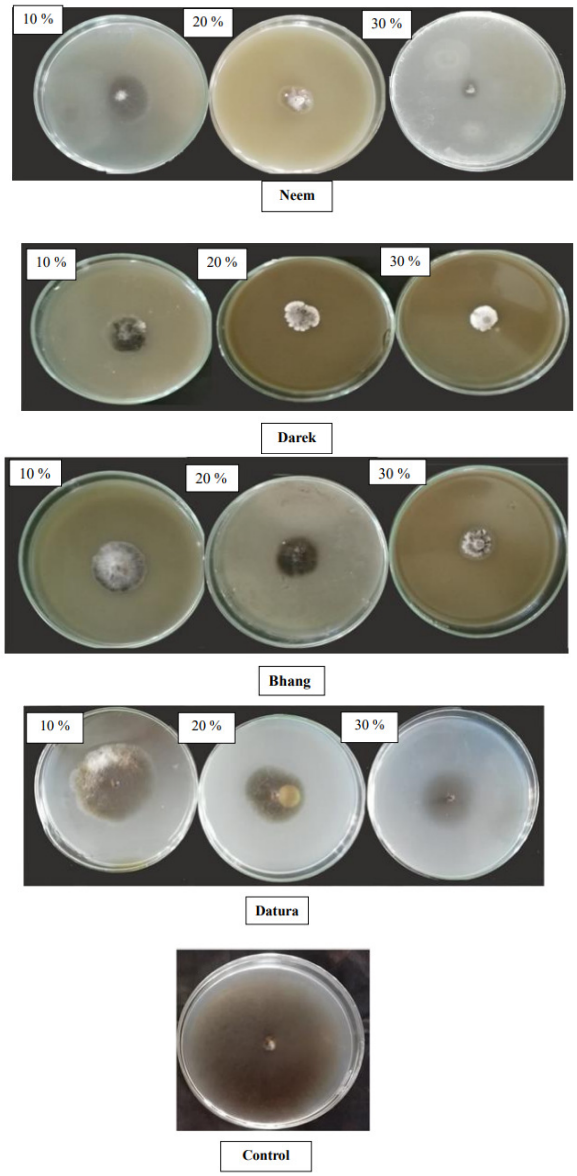

Plate 2. *In vitro* evaluation of leaf extracts of botanicals against *A. tagetica.*

Table 4: *In-vitro* evaluation of leaf extracts of botanicals against *Alternaria tagetica.*

Botanicals	Mycelial growth inhibition (%) at different concentrations (%)		
	10	20	30
Neem (*Azadirachta indica*)	71.56 (57.78)	78.22 (62.19)	81.56 (64.57)
Darek (*Azadirachta melia*)	70.33 (57.00)	77.22 (61.50)	80.11 (63.52)
Bhang (*Cannabis sativa*)	68.33 (55.76)	75.56 (60.38)	79.22 (62.89)
Datura (*Datura metel*)	67.11 (55.01)	73.56 (59.05)	78.33 (62.27)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	C.D. $_{p\geq0.05}$	S.E.(d)	
Botanicals (A)	1.113	0.379	
Concentration (B)	0.964	0.328	
Interaction	1.928	0.656	

**Values in the parentheses are angular transformed*

Neem was the most effective showing efficacy and least fungal growth was obtained at 30 per cent. However, with the increase in concentration, the efficacy of all the botanicals enhanced and consequently the rate of fungal growth was declined. Among other botanicals, Darek was highly effective and recorded minimum fungal growth (26.70, 20.50, 17.90 mm) at 10, 20, 30 per cent followed by Bhang, while Datura was least effective recording the maximum fungal growth (29.60, 23.80, 19.50 mm) at 10, 20 and 30 per cent respectively. When the rate of inhibition of fungal growth was compared over control, the maximum inhibition was observed in Neem (71.55, 78.22, 81.55 %), followed by Darek (70.33, 77.22, 80.11 %), Bhang (68.33, 75.55, 79.22 %) and minimum inhibition was observed in Datura (67.11, 73.55, 78.33 %) at 10, 20, 30 per cent respectively.

Our results were approved by Khanna and Chandra (1972); Kozmi *et al*. (1993); Shrinivas *et al.* (1998); Dubey (2001) also reported that *A. indica* in different forms is effective against *Alternaria* spp. while Gupta (2005) reported that volatile oils from *A. indica* and *Eucalyptus* sp. were more effective against *A. tagetica.*

Evaluation of different fungicides and botanicals against leaf spot and flower blight of marigold under field conditions. An experiment was conducted under field conditions to evaluate the performance of four fungicides and four botanicals against *A. tagetica.* The fungicides and botanicals were sprayed twice at fifteen day's intervals after appearance of disease using different concentrations. Observations on the disease severity were determined based on the percentage of disease severity. All the fungicides and botanicals were found to significantly reduced the leaf spot and flower blight infection as compared to the untreated control.

Data revealed that in fungicides, Azoxystrobin was most effective whereas in botanicals, Neem was the most effective in controlling disease infection. (Table 5) showed that the rate of increase of disease in untreated plots at 45, 60 days after transplanting was 36.33 and 46.66 per cent, respectively, while on the same dates of observations, per cent disease severity in azoxystrobin treated plot was 14.54 and 16.36 per cent, followed by mancozeb (18.20, 21.81%), copper oxychloride (25.45, 27.90%), carbendazim (27.30, 29.72%), Neem (28.88, 32.85%), Darek (31.11, 34.62 %), Bhang (32.40, 36.81%) and Datura (34.54, 39.40%).

Table 5: Evaluation of fungicides and botanicals against leaf spot and flower blight of marigold cultivar Pusa Basanti under field conditions.

Treatment *	Concentration (%)	45 DAT		60 DAT	
		Disease severity (%)	Disease reduction (%)	Disease severity (%)	Disease reduction (%)
Azoxystrobin	0.10	14.54 (22.40)	59.97	16.36 (23.85)	64.93
Carbendazim	0.10	27.30 (31.51)	24.85	29.72 (33.02)	36.30
Mancozeb	0.20	18.20 (25.24)	49.90	21.81 (27.82)	53.25
Copper oxychloride	0.30	25.45 (30.28)	29.94	27.90 (31.87)	40.20
Neem	30	28.88 (32.48)	20.50	32.85 (34.95)	29.59
Darek	30	31.11 (33.89)	14.36	34.62 (36.03)	25.80
Bhang	30	32.40 (34.68)	10.81	36.81 (37.34)	21.11
Datura	30	34.54 (35.98)	00.05	39.40 (38.86)	15.55
Control		36.33 (37.05)		46.66 (43.07)	
SE(d)±		0.282		0.338	
CD $_{p\geq0.05}$		0.853		1.022	

**Values in the parentheses are angular transformed*

DAT = Days after transplanting ; *1st spray = 30 DAT; 2nd spray = 45 DAT

The present findings are supported by Khanna and Chandra (1972); Kozmi *et al*. (1993); Shrinivas *et al.* (1998); Dubey (2001) also reported that *A. indica* in different forms is effective against *Alternaria* spp. while Gupta (2005) reported that volatile oils from *A. indica* and *Eucalyptus* sp. were more effective against *A. tagetica.* Tetrawal and Ral (2007) reported that mancozeb was highly effective against the *A. alternata* causing leaf blight of senna. Mancozeb, Bordeaux mixture and zineb effectively controlled the *A. sesammi* under field conditions (Samuel *et al*., 1971 ; Abhram *et al*., 1976). Srivastava and Gupta (1983) found that zinnia seeds treated with Dithane M-45 (mancozeb) reduced the infection caused by *A. zinniae* as well as improved the seed germination. Shinde *et al*. (2018) while evaluating different fungicides, botanicals and bio-agent under field conditions reported that Hexaconazole (0.1%) was found effective in reducing the percent disease index (32.15) on leaves and (33.76) on flower in all three sprays and getting higher yields (6.96 t/ha) followed by Mancozeb (0.2%) with PDI of (34.53) on leaves and (35.45) on flower with yield of (6.81 t/ha) as compared to control with (85.02 and 86.11 PDI) on leaves and flower, respectively, with yield of (4.26 t/ha). Further, they observed that among botanicals, Nimbicidin (0.5%) was effective in minimizing the per cent disease index (14.80) on leaves and (16.81) on flowers and yield (4.81 t/ha).

During field surveys and evaluation of cultivars, none of the marigold cultivar was found resistant or tolerant to leaf spot and flower blight disease from seedling to maturity stage of marigold crop. To have good crop with minimum disease, a proper attention on control measures is most important. Since the vulnerable period to attack by the disease is maximum at mid growth stage usually 45-90 days after transplanting, as such, the chemical treatment with Azoxystrobin at 0.1 per cent, mancozeb at 0.2 per cent and neem at 30 per cent

with growing resistant cultivars is necessary for the proper management of the disease.

CONCLUSIONS

In conclusion, the study underscores the economic significance of Alternaria leaf spot and flower blight caused by *Alternaria tagetica* in marigold production. The *in-vitro* evaluation of four fungicides and four botanicals revealed substantial inhibition of *A. tagetica* mycelial growth, even at minimal concentrations. Notably, Azoxystrobin at 75ppm emerged as the most effective systemic fungicide, while Mancozeb at 750ppm stood out among non-systemic fungicides. Neem exhibited remarkable efficacy at 30 per cent concentration among the botanicals. Field trials confirmed the practical utility of Azoxystrobin at 0.1 per cent, surpassing other treatments, with Mancozeb following closely, demonstrating promising disease reduction in real-world conditions at 64.93 and 53.25 per cent, respectively.

FUTURE SCOPE

By using proper management strategies including chemical as well as eco-friendly disease management, the production and productivity of a particular area enhances hence, flourish and generate tremendous money and employment.

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Conflict of Interest. None.

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