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Management of *Alternaria alternata* of Tomato (*Lycopersicon esculentum Mill.)* through Plant Extract and Fungicides *in vitro* and Natural Condition

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A B S T R A C T

The experiment was conducted at Department of Plant Pathology, S.K.N. College of Agriculture, Jobner (Rajasthan). *Alternaria alternata* was isolated from leaves of tomato and observed to be pathogenic under artificial conditions. It is cause heavy yield loss in Rajasthan as well as in India. An attempt was more find out the efficacy of different plant extracts and fungicides were against in *Alternaria alternata in vitro* and *in vivo* conditions. Among five Plant extracts garlic was found most effective followed by neem and among six fungicides propiconazole was found most effective followed by trifloxystrobin+tebuconazole against *Alternaria alternata in vitro* conditions. In potted plant minimum disease intensity were obtained in garlic and propiconazole and followed by neem and trifloxystrobin+tebuconazole. Garlic and propiconazole were found effective in management of leaf blight of tomato by *Alternaria alternata in vitro and in vivo* conditions.

Introduction

Tomato (*Solanum lycopersicum* L., syn. = *Lycopersicon esculentum* Mill.) belongs to the family *Solanaceae* and is one of the most remunerable and widely grown vegetables in the world. Among the vegetables, tomato ranks next to potato in world acreage and first among the processing crops.

Tomato is grown for its edible fruits, which can be consumed either fresh or in processed form and is a very good source of vitamin A,B,C and minerals. Tomato cultivation has become more popular since mid nineteenth century because of its varied climatic adaptability and high nutritive value. Tomato is being exported in the form of whole fruits, paste and in canned form to West Asian countries, U.K., Canada and USA.

Being the world's fourth most cultivated crop, with a production of 130 million tonnes and area of 5.2 million hectares, the tomato is an indispensible vegetable crop world over and, of course, for India. India is the third largest producer of tomato in the world after USA and China having an area of 0.88 million hectares with a production of 187.35 lakh tonnes during 2013-14 (Anonymous, 2014). In Rajasthan, tomato is cultivated over an area of 0.017 million hectares with an annual production of 0.817 lakh tonnes (Anonymous, 2014).

In India, tomato crop is mainly grown in the states of Andhra Pradesh, Odisha, West Bengal, Karnataka, Bihar, Gujarat, Tamilnadu, U.P, Rajasthan etc**.**

In Rajasthan, tomato crop is mainly cultivated in Jaipur, Dausa, Alwar and Chittorgarh districts.

There are several diseases on tomato caused by fungi, bacteria, viruses, nematodes and abiotic factors (Balanchard, 1992). Among the fungal diseases, early blight also known as target spot disease incited by *Alternaria solani* (Ellis and Martin) Jones and Grout, is one of the world's most catastrophic diseases. The causal organism is air borne and soil inhabiting and is responsible for early blight, collar rot and fruit rot of tomato (Datar and Mayee, 1981).

It is very difficult to manage *Alternaria solani*, due to its broad host range, extreme variability in pathogenic isolates and prolonged active phase of the disease cycle. A coefficient of disease index of 71.66 per cent caused 78.51 per cent loss in fruit yield under severe epidemic (Datar and Mayee, 1981). The yield loss of tomato fruits was 78 per cent recorded at 72 per cent disease intensity by *A. solani* and each 1 per cent increase, reduced tomato yield by 1.36 per cent (Datar and Mayee, 1985). The disease appears on leaves, stems, petioles, twigs and fruits under favorable conditions resulting in defoliation, drying off of twigs and premature fruits drop and thus causing loss from 50 to 86 per cent in fruit yield (Mathur and Shekhawat, 1986). Pathogen also causes fruit rot in pre-harvest and post-harvest stages. Thus, infected fruits are disqualified in the market. *A. solani* is also one of the most common causes of seedling blight or damping off in tomato, causing dark lesions on the rootlets (Bose *et al*., 2002).

Bessadat *et al.,* (2014) reported 46-90 per cent blight intensity in tomato due to *Alternaria alternata* in Algeria.

Present investigation was carried out to test the efficacy of plant extracts and fungicides against leaf blight of tomato incited by *Alternaria alternata.*

Materials and Methods

Efficacy of different plant extracts and fungicides were evaluated against *Alternaria alternata*

Efficacy of plant extracts against *Alternaria alternata* (*in vitro)*

In recent years, many phyto-extracts are being used as fungitoxicants for the management of various plant diseases. The present investigation was carried out using following five natural phyto-extracts to see their antimycotic behaviour on the growth of *Alternaria alternata* following Poisoned Food Technique.

Common name	Botanical name	Plant part used	Concentration (%)
Neem	*Azadirachta indica*	leaves	5, 10
Turmeric	*Curcuma longa*	Rhizome	5, 10
Garlic	*Allium sativum*	Cloves	5, 10
Alstonia (devil's tree)	*Alstonia scholaris*	Leaves	5, 10
Thor	*Euphorbia caducifolia*	Stem	5, 10
Control	-	-	-

The effect of each plant extract was tested at two different concentrations (5 & 10%) following the method suggested by Singh and Majumdar (2001) with slight modifications. To get these, the required plant part was thoroughly washed with sterilized water and ground separately in electric grinder using equal amount of sterilized distilled water (i.e. 1:1 ratio, w/v). The mixture was squeezed with double layered sterilized cheese cloth. The extracts thus obtained were considered as of 100 per cent concentration.

Required quantity of each plant extract (i.e. stock solution) was mixed thoroughly in melted PDA, to get desired concentration, just before pouring in sterilized 9 cm diameter glass Petridishes and was allowed to solidify for 12 hours. Each plate was inoculated with 5 mm disc of mycelial bit taken with the help of sterilized cork borer from the periphery of 7 days old culture of *A. alternata* growing on PDA. The inoculated petridishes were incubated at 25±1ºC. Three petridishes were used for each treatment serving as three replications. A control was also maintained where medium was not supplemented with any plant extract. The experiment was conducted in completely Randomized Design (CRD). Colony diameter (two diagonals) was measured after 7 days of incubation. Per cent growth inhibition was calculated by Vincent's (1947) formula as follows:

$$\text{Per cent growth inhibition} = \frac{C - T}{C} \times 100$$

Where,
C = diameter of the colony in check (average of both diagonals)
T = diameter of colony in treatment (average of both diagonals)

Efficacy of fungicides against *Alternaria alternata* (*in vitro*)

Efficacy of six systemic and non-systemic fungicides carbendazim + mancozeb, azoxystrobin, mancozeb, haxaconazole, trifloxystrobin + tebuconazole and propiconazole against mycelial growth of *A. alternata* was tested by Poisoned Food Technique (Schmitzs 1930). Three different concentrations *viz.*, 100, 300 and 500 ppm of each fungicide was evaluated. Required quantity of each fungicide was added separately to sterilized medium, mixed thoroughly and poured in sterilized 9 cm diameter glass Petriplates and allowed to solidify. Three replications were maintained for each treatment. A control was also maintained where medium was not supplemented with any fungicides. Each plate was inoculated with 5 mm discs with the help

of sterilized cork borer from the edge of the fungal culture and incubated at 25±1ºC for 7 days. The linear growth of the test fungus was recorded and per cent growth inhibition was calculated by Vincent's (1947) formula as mentioned above under 3.4.1.1.

Efficacy of plant extracts and fungicides against *Alternaria alternata* (*in vivo*)

Plant extracts and fungicides, which proved efficacious *in vitro* were also evaluated by spraying, on the susceptible variety (Arka Vikas) in mini plots (1 x 1 m) with three replications. Inoculation was done 30 days after transplanting (DAT) with spore-cum mycelial suspension of *Alternaria alternata* (1 x 10^3 spore/ml). To prepare the spore suspension of *A. alternata* spores obtained from 10 days old culture on PDA was suspended in sterilized water and diluted to obtain spore suspension of (1 x 10^3 spore / ml) as viewed under light microscope. By covering inoculated plants with polythene bags and spraying sterilized water frequently, high humidity was maintained. Five days after inoculation (i.e. 35 days after transplanting), plants were sprayed with respective plant extracts and fungicides and second spray was applied at 50 days after transplanting. Plant extracts and fungicides and their concentration used were as follows:

Effective plant extracts and fungicides with their concentration.

For calculating per cent disease intensity (PDI), observations of above experiment *viz.*, plant extracts and fungicides were recorded as per cent leaf area covered by leaf spot at 60 and 90 days after transplanting.

TREATMENT	CONCENTRATION (%)
Neem	10
Garlic	10
Propiconazole	0.1
Trifloxystrobin + Tebuconazole	0.1
Control	-

Results and Discussion

Efficacy of plant extracts *in vitro*

The efficacy of five plant extracts (Table 1, Fig 1) was tested *in vitro* at two concentrations viz., 5 and 10 per cent against *Alternaria alternata* on PDA by Poisoned Food Technique. Among five plant extracts, extract of garlic cloves was found most effective in inhibiting mycelial growth (50.20 and 65.45%) of *Alternaria alternata* at 5 and 10 per cent, respectively followed by neem (45.40 and 55.12%) over control. Extracts of thor (40.13 and 45.18%), turmeric (25.25 and 40.23%) and alstonia (20.30 and 38.18%) were found least effective in inhibiting mycelial growth of *Alternria alternata* over control. All the concentrations (5 and 10%) of all the tested plant extracts were found significantly superior with each other.

The efficacy of garlic and neem as antifungal substances against various plant pathogenic fungi has also been investigated by Singh and Majumdar (2001) and Choudhary *et al.*, (2003).

Efficacy of fungicides *in vitro*

The efficacy of six fungicides (Table 2 Fig 2) was tested *in vitro* at three concentrations viz. 100, 300 and 500 ppm against *A. alternata* on PDA by Poisoned Food Technique. Among six fungicides, propiconazole was found most effective in inhibiting mycelial growth (94.00, 100 and 100%) of *A. alternata* at 100, 300 and 500 ppm, respectively followed by trifloxystrobin+tebuconazole (75.00, 90.11 and 95.88%) over control. Fungicides, hexaconazole (70.00, 76.33 and 85.44%), carbendazim + mancozeb (64.25, 69.15 and 80.10%), azoxystrobin (57.00, 62.33 and 70.00%) and mancozeb (55.70, 59.00 and 65.66%) were found least effective in inhibiting mycelial growth over control. All

the concentrations (100, 300 and 500 ppm) of tested fungicides were found significantly superior with each other except propiconazole at 300 and 500 ppm.

Importance of propiconazole and trifloxystrobin+tebuconazole were found highly effective in inhibiting mycelial growth of *Alternaria alternata* in laboratory reported by kumar and singh (1997), Kamble *et al.*, (2000) and Rao and Rao (2002).

Table.1 Fungitoxicity of different plant extracts against *Alternaria alternata* by Poisoned Food Technique after 7 days of incubation at 25 ± 1^0C

Common name of plant	**Scientific name**	**Part used**	**Per cent inhibition of mycelial growth at different concentration***		
			5%	10%	Mean
Garlic	*Allium sativum*	Clove	50.20	65.45	57.83
			(45.11)	(54.00)	
Neem	*Azadirachta indica*	Leaf	45.40	55.12	50.26
			(42.36)	(47.94)	
Thor	*Euphorbia caducifolia*	Stem	40.13	45.18	42.66
			(39.31)	(42.23)	
Turmeric	*Curcuma longa*	Rhizome	25.25	40.23	32.74
			(30.17)	(39.37)	
Alstonia	*Alstonia scholaris*	Leaf	20.30	38.18	29.24
			(26.78)	(38.16)	
Control	-	-	0.00	0.00	0.00
			(0.00)	(0.00)	
				SEm±	CD (p=0.05)
			P	0.36	1.05
			C	0.21	0.60
			PxC	0.50	1.48

Average of three replications
Figures given in parentheses are angular transformed values

Table.2 Efficacy of fungicides against *Alternaria alternata* by Poisoned Food Technique after 7 days of incubation at 25 ± 1^{0}C

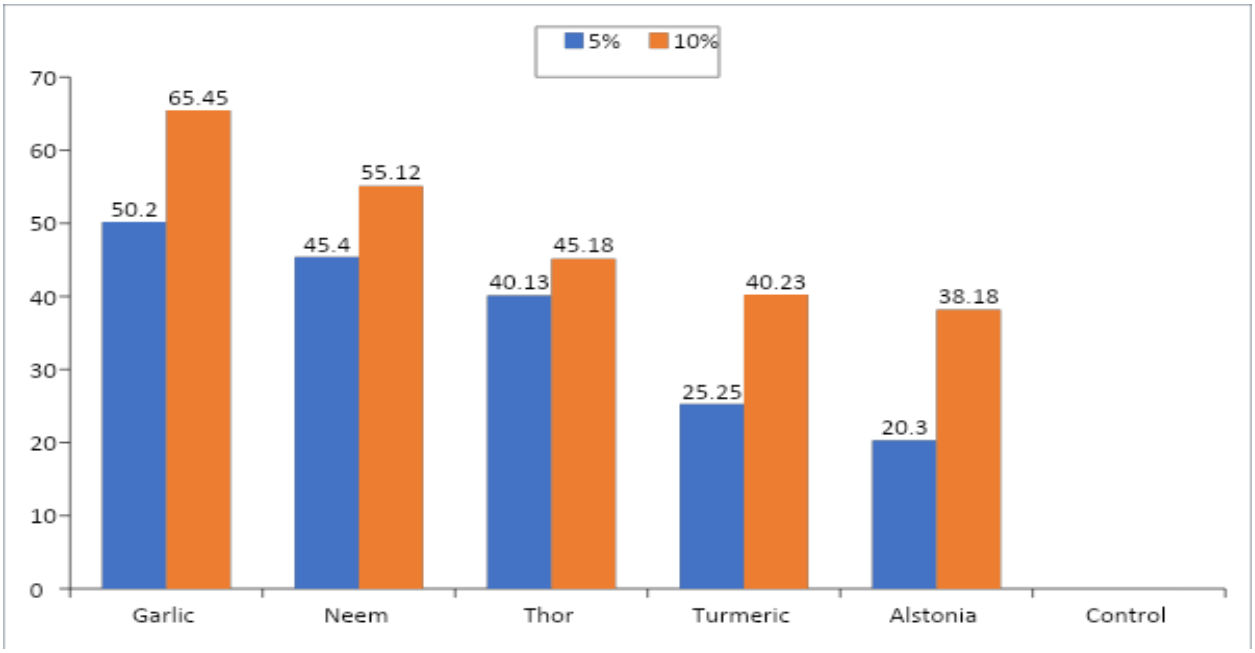
Fungicides		**Per cent inhibition of mycelial growth at various concentration* (ppm)**			
Common name	**Trade name**	**100**	**300**	**500**	**Mean**
Carbendazim+ mancozeb	Sprint	64.25 (53.28)	69.15 (56.26)	80.10 (63.51)	71.17
Azoxystrobin	Amistar	57.00 (49.02)	62.33 (52.14)	70.00 (56.79)	63.11
Mancozeb	Indofil M-45	55.70 (48.27)	59.00 (50.18)	65.66 (54.13)	60.12
Hexaconazole	Sitara	70.00 (56.79)	76.33 (60.89)	85.44 (67.57)	77.26
Trifloxystrobin + tebuconazole	Nativo	75.00 (60.00)	90.11 (71.67)	95.88 (78.29)	87.00
Propiconazole	Tilt	94.00 (75.82)	100.00 (90.00)	100.00 (90.00)	98.00
Control	-	0.00	0.00	0.00	-
		(0.00)	(0.00)	(0.00)	
				SEm±	CD (p=0.05)
			F	0.69	1.96
			C	0.49	1.39
			FxC	1.19	3.40

Average of three replications
Figures given in parentheses are angular transformed values

Table.3 Effect of plant extracts and fungicides on Alternaria leaf blight of tomato (*in vivo*)

Treatments	**Dose. (%)**	**PDI***		**Per cent disease control**	
		60 DAT	**90 DAT**	**60 DAT**	**90 DAT**
Garlic	10	11.80	32.60	38.22	49.85
		(20.09)	(34.82)		
Neem	10	13.50	35.00	29.32	46.15
		(21.56)	(36.27)		
Propiconazole	0.1	5.00	23.50	73.82	63.85
		(12.92)	(29.00)		
Trifloxystrobin + Tebuconazole	0.1	7.80	27.90	59.16	57.08
		(16.22)	(31.88)		
Control	-	19.10	65.00	-	-
		(25.91)	(53.73)		
SEm±		0.17	0.55		
CD (p=0.05)		0.51	1.64		

* Average of three replications
Figures given in parentheses are angular transformed values
PDI = Per cent disease intensity, DAT = Days after transplanting



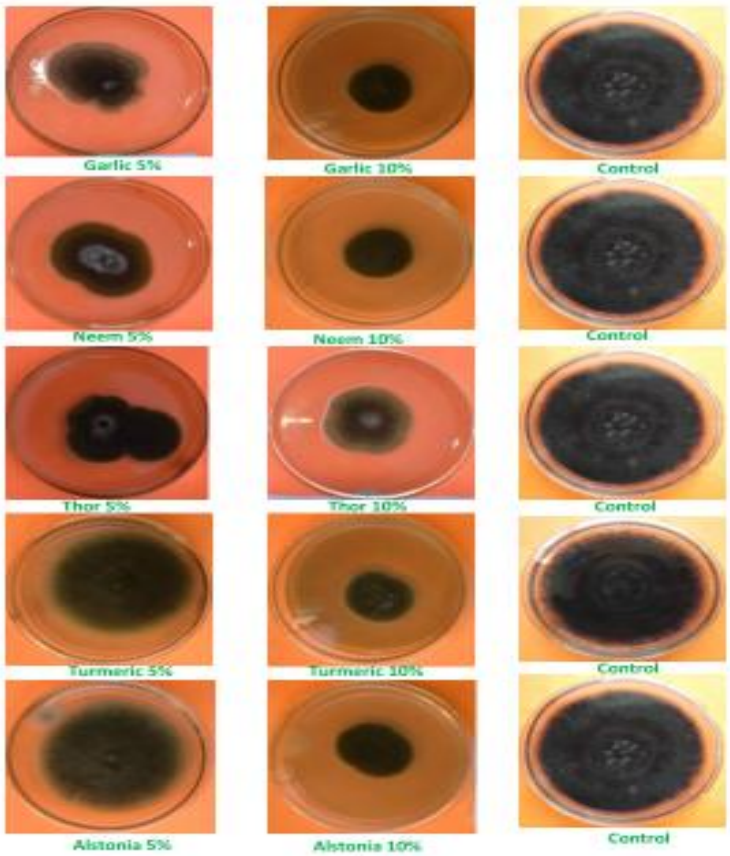


Plate.3 Fungitoxicity of different plant extracts against *Alternaria alternate (invitro)*

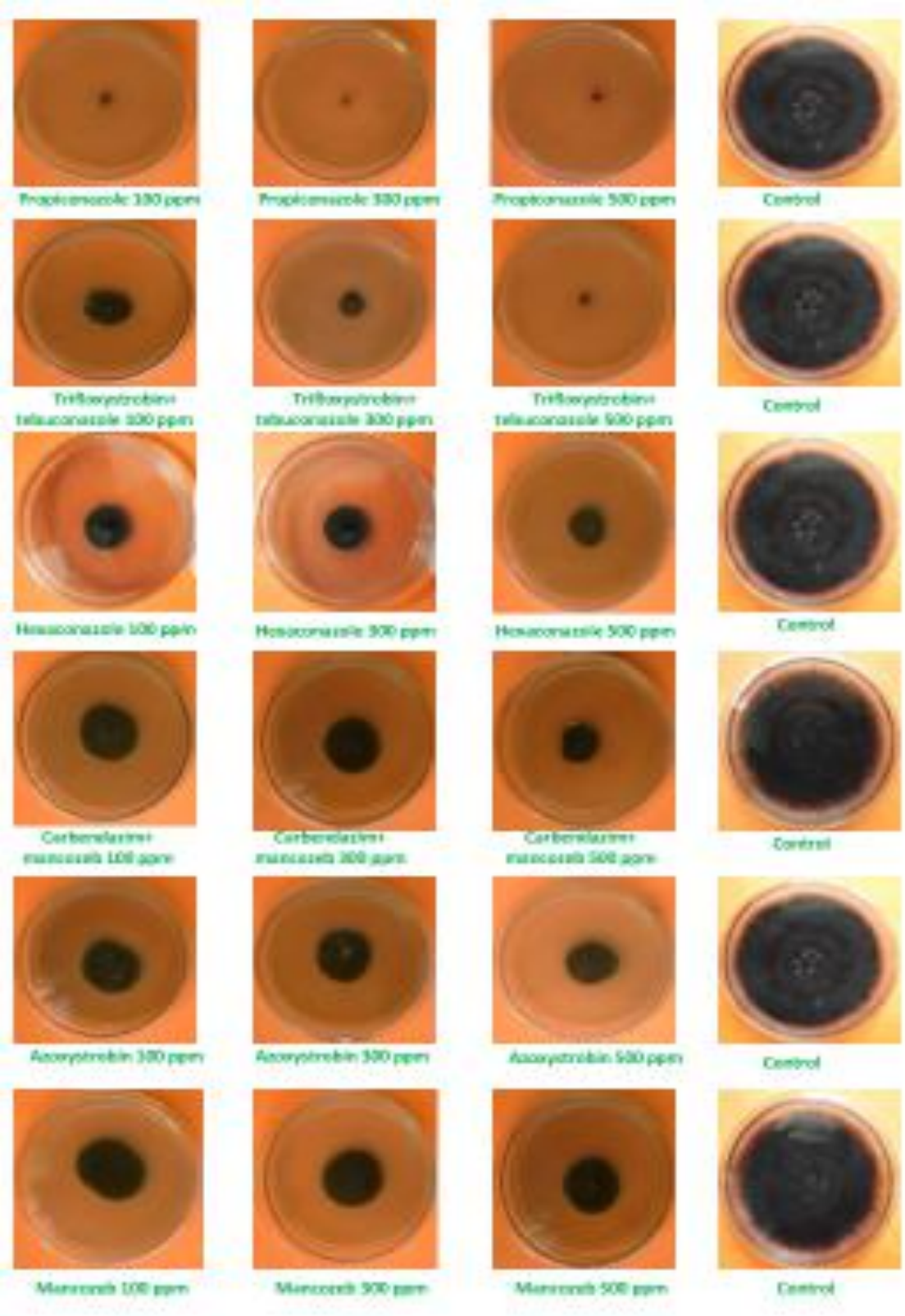
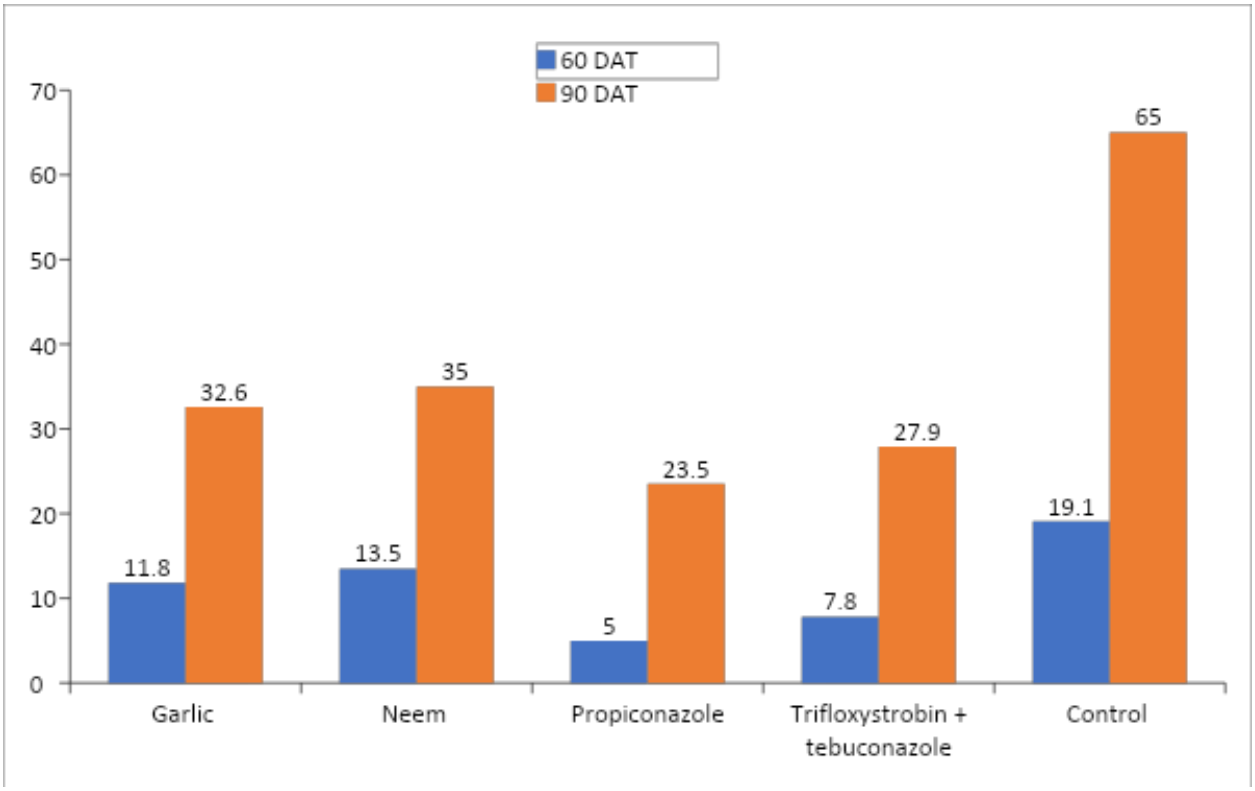
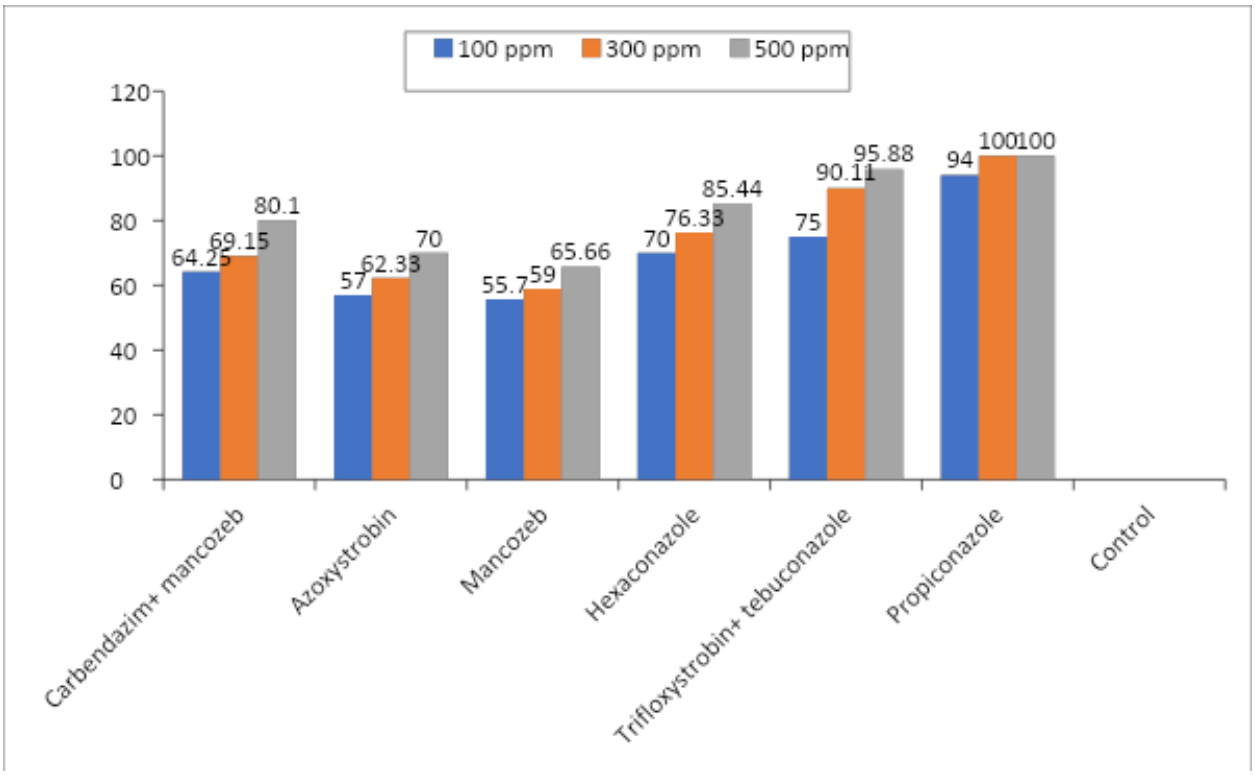


Plate.4 Efficacy of different fungicides against *Alternaria alternate (invitro)*


60 DAT
90 DAT
70
60
50
40
30
20
10
0
11.8
32.6
13.5
35
5
23.5
7.8
27.9
19.1
65
Garlic
Neem
Propiconazole
Trifloxystrobin + tebuconazole
Control


100 ppm
300 ppm
500 ppm
120
100
80
60
40
20
0
64.25
69.15
80.1
57
62.33
70
55.7
59
65.66
70
76.33
85.44
75
90.11
95.88
94
100
100
Carbendazim+ mancozeb
Azoxystrobin
Mancozeb
Hexaconazole
Trifloxystrobin+ tebuconazole
Propiconazole
Control

Efficacy of plant extracts and fungicides in reducing disease intensity (*in vivo*)

Plant extracts and fungicides which were found most effective in *in vitro* were also tested as foliar spray in mini plots against *Alternaria alternata* and these were garlic, neem, propiconazole and trifloxystrobin + tebuconazole.

The results depicted in Table 3 and Fig. 3 revealed that all plant extracts & fungicides were found significantly superior over control in reducing disease intensity at 60 and 90 days after transplanting (DAT). Minimum disease intensity was recorded with propiconazole (5.00 and 23.00%) followed by trifloxystrobin + tebuconazole (7.80 and 27.90%), garlic (11.80 and 32.60%) and neem (13.50 and 35.00%) over control (19.10 and 65.00%) at 60 and 90 days after transplanting (DAT), respectively. At 90 days after transplanting (DAT), each treatment differed significantly except garlic and neem which were at par to each other. Present results are in accordance with the results of Datar (1992), Bai (1992), Chattopadhyay (2001) and Singh and Majumdar (2001). They reported many plant extracts and fungicides in controlling Alternaria blight of Safflower, tomato and brassicas in field as well as in laboratory

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