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Screening of fungicides against *Alternaria alternata* (Fr.) Keissler *In vitro* condition

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Abstract

Exploration on leaf spot disease (*Alternaria alternata* (Fr.) Keissler) of brinjal (*Solanum melongena* L.) under South Gujarat condition was carried out to find suitable management strategies. Ten fungicides from systemic and non-systemic groups were evaluated at three different concentrations by poisoned food technique *in vitro* for their efficacy against *A. alternata*. The results revealed that out of all ten fungicides screened at three concentrations, four fungicides were found significantly superior in inhibiting the mycelial growth and sporulation of *A. alternata*. However, cent per cent inhibition of *A. alternata* was recorded with Propiconazole (Tilt), Difenoconazole (Score) and Hexaconazole (Contaf) at all three concentrations (250, 500, 1000 ppm) tried and Mancozeb (Dithane M-45) at 2500 ppm. The next best in order of efficacy were Carbendazim (12%) + Mancozeb (63%) (Sixer 75 % WP) at 2500 ppm (93.05 %) and 2000 ppm (87.50 %). Copper oxychloride (Blitox) at 2500 ppm (86.11%), followed by, Propineb (Antracol) at 1000 ppm (77.77 %) and Tridemorph (Calixin) at 1000 (74.07%). The spore formation was totally inhibited by Propiconazole (Tilt), Difenoconazole (Score), Hexaconazole (Contaf), Copper oxychloride (Blitox) and Mancozeb (Dithane M-45) at all three concentrations. The next best in order of merit was Propineb (Antracol) at all three concentrations.

Keywords: Fungicides, brinjal, *A. alternata*

Introduction

In India, brinjal is consumed as cooked vegetable in various ways. It has nutritive value in human diet, because it contains about 1.4 g proteins, 4.0 g carbohydrates, 0.3 g fat, 18 mg calcium, 2.0 mg potassium, and 0.9 mg iron per 100 g of edible portion. It also provides vitamins like A, B and C. (Choudhary and Gaur, 2009) [5].

Besides having nutritive values, it also possesses medicinal properties. The white brinjal is said to be good food for diabetic patients (Choudhary, 1976) [4]. It can be used as excellent remedy of liver suffering patients (Chauhan, 1981) [3]. The fruit is used as a cure for toothache. The green leaves of brinjal are the main source of Vitamin-C. The seeds of brinjal are used as a stimulant. (Nadkarni, 1972) [7].

It was reported that on an average, the oblong-fruited eggplant cultivars are rich in total soluble sugars, where as the long-fruited cultivars contain a high free reducing sugars, anthocyanin, phenols, glycoalkaloids (such as solasodine), dry matter and a low level protein. Bitterness in eggplant is due to the presence of glycoalkaloids, which are of wide occurrence in plants of Solanaceae family. The glycoalkaloid contents in the Indian commercial cultivars vary from 0.37 to 4.83 mg/100 g fresh weight. Generally the high content of glycoalkaloids (20mg/100 g fresh weight) produce a bitter taste and off flavor (Bajaj, *et al.*, 1979) [2].

Among all the fungal diseases, *Alternaria* leaf spot, *Alternaria* leaf blight and fruit rot diseases are of regular occurrence in moderate to severe proportion in India and causes extensive damage to the quality of fruits (Pandey and Vishwakarma (1999) [8]. The important pathogenic fungi *Viz*; *Alternaria solani*, *Phomopsis vexans*, *Alternaria alternata* and *Fusarium oxysporum* which causes leaf spot, fruit rot, blight, wilt and discoloration of seeds in brinjal crop, respectively. Infected seeds caused reduction in seed germination and yield loss up to 30-50 Per cent in the year 2001-2002. (Thippeswamy *et al.*, 2005) [11]. Cool and humid weather, coupled with cloudiness which favors the occurrence and spread of the disease. When humid conditions prevailing at ground level, lower leaves are first attacked and infections spread to the upper leaves and fruits. The disease causes characteristics leaf spots with concentric rings. The spots are mostly irregular and coalesce to cover a large leaf area.

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Considering the seriousness of the problem, the present exploration of Screening of fungicides against *Alternaria alternata* (Fr.) Keissler *in vitro* condition was carried out.

Materials and Methods

Ten fungicides belonging to different chemical groups at three different concentrations as listed in table no. 1. Were tested for their efficacy *in vitro* against *A. alternata* by poisoned food technique. The required quantities of each test fungicides were incorporated in conical flask containing 100 ml molten RA medium so as to get required concentration in parts per million (ppm). The flask containing poisoned medium was well shaken to facilitate uniform mixing of fungicides and 20 ml was poured in each sterilized Petri-plates. On solidification of the medium, the plates were inoculated in the centre by placing 5mm diameter mycelial culture block, cut aseptically with the help of cork borer from 10 days old pure culture of *A. alternata* grown on PDA. Three repetitions were kept for each

concentration of respective fungicide. The inoculated plates were incubated at $27\pm 2^{\circ}\text{C}$ temperature. The required concentration in ppm was calculated based on active ingredient present in a formulation. The colony diameter of the fungus was recorded from three repetitions periodically. The per cent growth inhibition over control was calculated by using formula as given below:

$$\text{PGI} = \frac{100 (\text{DC}-\text{DT})}{\text{DC}}$$

Where,

PGI = Per cent growth inhibition

DC= Average diameter of mycelial colony of control set (mm)

DT= Average diameter of mycelial colony of treated set (mm)

Table 1: Details of fungicides screened against *A. alternata in vitro*

S. No	Trade name	Technical name	Chemical name	Concentration (ppm)			Source (Manufacture)
				1 st	2 nd	3 rd	
1	Dithane M-45 (75% WP)	Mancozeb	Zinc ion and manganese ethylene bisdithiocarbamate	1000	1500	2000	Indofil Chemicals Ltd., Mumbai
2	Blitox (50 % WP)	Copper oxychloride	Basic cupric chloride	1000	1500	2000	Sandoz (India) Ltd., Mumbai
3	Kavach (75 % WP)	Chlorothalonil	Tetra chloro isopathalonitrite	1000	1500	2000	Sandoz (India) Ltd., Mumbai
4	Sixer (75 % WP)	Carbendazim (12 %) + Mancozeb (63 %)	2 (methoxy-carbamoyl)-benzimidazole Zinc ion and manganese ethylene bisdithio- carbamate	1000	1500	2000	Northern Minerals Ltd., Haryana
5	Antracol (70 % WP)	Propineb	Zinc propylenebisdithio-carbamate	250	500	1000	Bayer (India) Ltd., Mumbai
6	Calixin (80 % EC)	Tridemorph		250	500	1000	
7	Score (25 % EC)	Difenconazole	Triazole compound	250	500	1000	Novartis (India) Ltd., Mumbai
8	Contaf (5 % EC)	Hexaconazole	(RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazole-1YL) hexan-2-0)	250	500	1000	Rallis (India) Ltd., Ahmedabad
9	Tilt (25% EC)	Propiconazole	1-[2-(2,4-dichlorophenyl)- 4-propyl-1,3-dioxalan-2,1,methyl] 1,2,4-triazole	250	500	1000	Novartis (India) Ltd., Mumbai
10	Bavistin (50% WP)	Carbendazim	2 (methoxy-carbamoyl)-benzimidazole	250	500	1000	BASF (India) Ltd., Mumbai

Results and Discussion

Ten fungicides from systemic and non-systemic groups were evaluated at three different concentrations by poisoned food technique *in vitro* for their efficacy against *A. alternata*. The

results presented in Table - 2 and depicted in Fig - 1 and Plate - 1 indicated that fungicides have varied efficacy against *A. alternata*.

Table 2: Screening of different fungicides against *A. alternata*

S. No	Technical and trade name of fungicide	Conc. (ppm) Tried	Average colony diameter (mm)	Per cent growth inhibition over control	Sporulation
1	Propiconazole (Tilt 25% EC)	250	0.71 * (0.00)**	100.00	-
		500	0.71 (0.00)	100.00	-
		1000	0.71 (0.00)	100.00	-
2	Hexaconazole (Contaf 5% EC)	250	0.71 (0.00)	100.00	-
		500	0.71 (0.00)	100.00	-
		1000	0.71 (0.00)	100.00	-
3	Difenoconazole (Score 25% EC)	250	0.71 (0.00)	100.00	-
		500	0.71 (0.00)	100.00	-
		1000	0.71 (0.00)	100.00	-
4	Mancozeb (Dithane M-45 75% WP)	1500	3.67 (13.00)	81.94	-
		2000	3.53 (12.00)	83.33	-
		2500	0.71 (0.00)	100.00	-
5	Carbendazim (12%) + Mancozeb (63%) (Sixer 75% WP)	1500	4.94 (24.00)	66.66	++
		2000	3.07 (9.00)	87.50	+
		2500	2.34 (5.00)	93.05	-

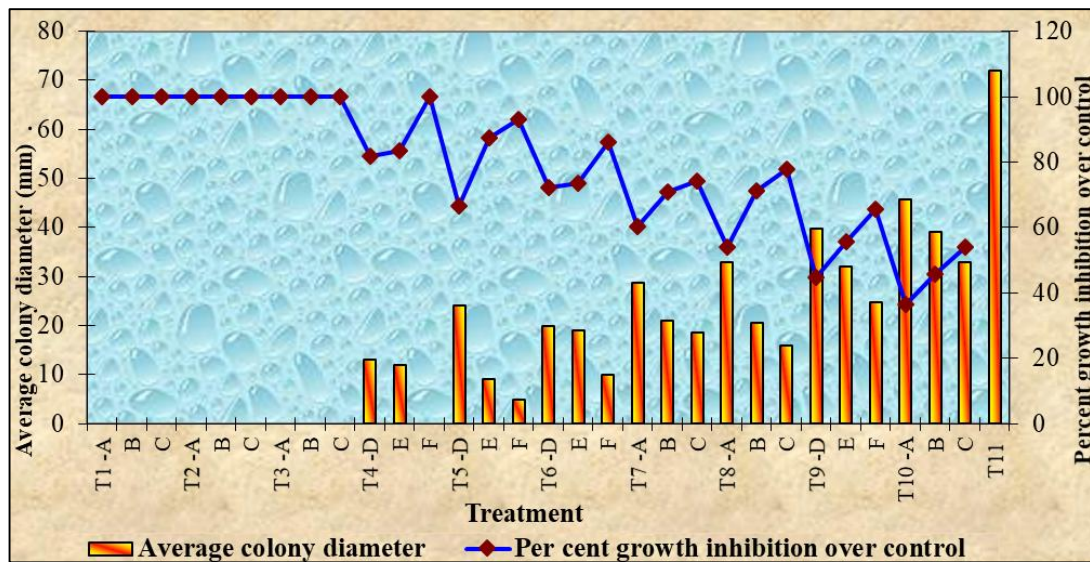
6	Copper oxychloride (Blitox 50% WP)	1500	4.52 (20.00)	72.22	-
		2000	4.41 (19.00)	73.61	-
		2500	3.24 (10.00)	86.11	-
7	Tridemorph (Calixin 80% EC)	250	5.40 (28.66)	60.18	++
		500	4.63 (21.00)	70.83	++
		1000	4.37 (18.66)	74.07	++
8	Propineb (Antracol 70% WP)	250	5.78 (33.00)	54.16	+
		500	4.59 (20.66)	71.29	+
		1000	4.06 (16.00)	77.77	+
9	Chlorothalonil (Kavach 75% WP)	1500	6.33 (39.66)	44.90	++
		2000	5.70 (32.00)	55.55	++
		2500	5.01 (24.66)	65.74	++
10	Carbendazim (Bavistin 50% WP)	250	6.79 (45.66)	36.57	+++
		500	6.28 (39.00)	45.83	+++
		1000	5.78 (33.00)	54.16	++
11	Control	-	8.51 (72.00)	-	++++
	S.Em ±		0.04		
	C.D. at 5%		0.13		
	C.V. %		2.28		

* Figures are SQR + 0.5 transformed values

** Figures in parenthesis are re-transformed values

Sporulation (No. of conidia / microscopic field) =

--- = No sporulation	++ =	Moderate (6- 15)
+ = Poor (below 5)		
+++ = Good (16-20)	++++ =	Excellent (above 30)



T1 = Propiconazole T2 = Hexaconazole T3 = Difenconazole
 T4 = Mancozeb T5 = Carbendazim (12%) + Mancozeb (63%)
 T6 = Copper oxychloride T7 = Tridemorph T8 = Propineb
 T9 = Chlorothalonil T10 = Carbendazim T11 = Control
 Concentration (ppm):- A = 250, B = 500, C = 1000, D = 1500, E = 2000, F = 2500.

Fig 1: Screening of fungicides against *A. alternata*

All the fungicides screened at three concentrations were found significantly superior in inhibiting the mycelial growth and sporulation of *A. alternata*. However, cent per cent inhibition of *A. alternata* was recorded with Propiconazole (Tilt), Difenconazole (Score) and Hexaconazole (Contaf) at all three concentrations (250, 500, 1000 ppm) tried and Mancozeb (Dithane M-45) at 2500 ppm.

The next best in order of efficacy were Carbendazim (12%) + Mancozeb (63%) (Sixer 75 % WP) at 2500 ppm (93.05 %) and 2000ppm (87.50 %). Copper oxychloride (Blitox) at 2500 ppm (86.11%), followed by, Propineb (Antracol) at 1000 ppm (77.77 %) and Tridemorph (Calixin) at 1000 (74.07%). While Chlorothalonil (Kavach) and Carbendazim (Bavistin) were moderately effective in growth inhibition as compared to other fungicides at all three concentrations tried. The spore

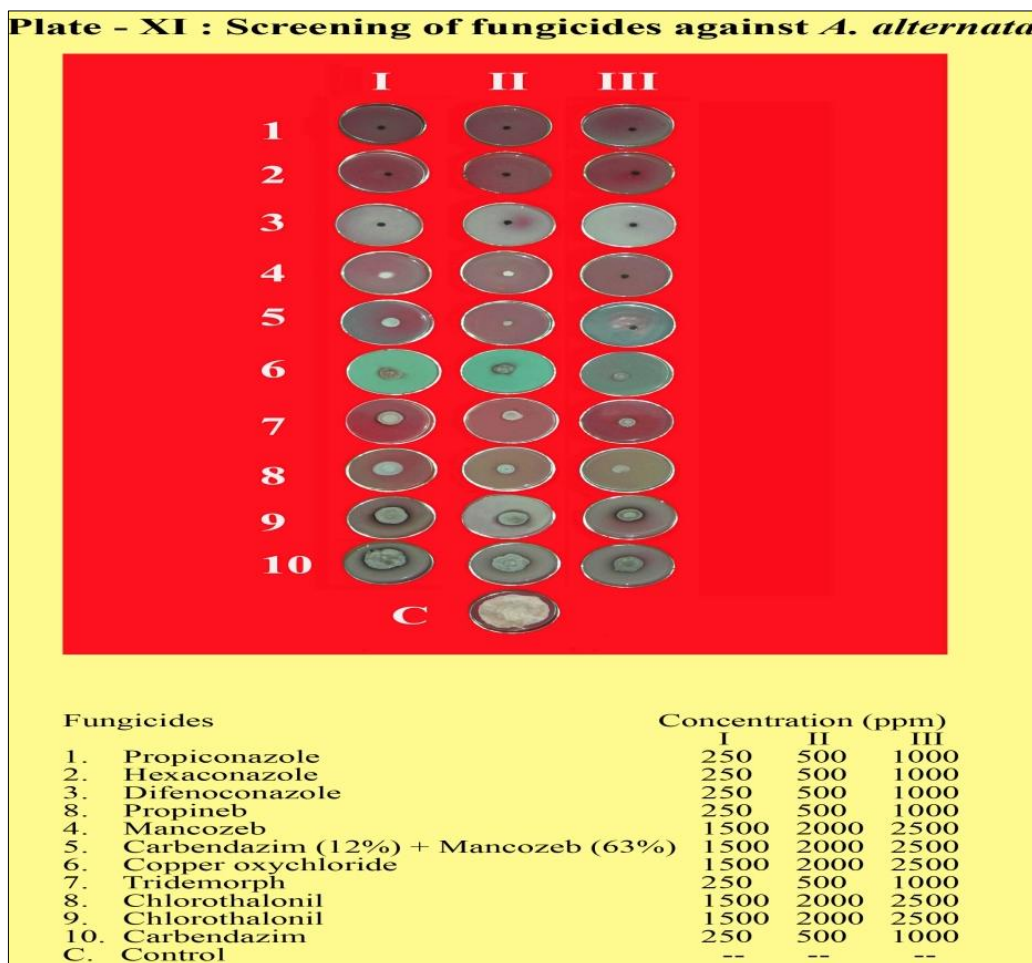
formation was totally inhibited by Propiconazole (Tilt), Difenconazole (Score), Hexaconazole (Contaf), Copper oxychloride (Blitox) and Mancozeb (Dithane M-45) at all three concentrations. The next best in order of merit was Propineb (Antracol) at all three concentrations.

It is evident from this result that, the growth inhibition increase with an increased in the concentration of chemicals. Propiconazole (Tilt), Difenconazole (Score) and Hexaconazole (Contaf) followed by, Mancozeb (Dithane M-45) and Carbendazim (12%) + Mancozeb (63%) (Sixer) were significantly superior at all three concentrations over rest of the fungicides tested.

Earlier, Propiconazole (Sharma *et al.*, 2002)^[9], Hexaconazole (Singh and Singh 2006), Propiconazole, Difenconazole and Hexaconazole (Akbari and Parakhia., 2007)^[11] and Mancozeb

(Lalesh Kumari *et al.*, 2006) ^[6], reported the most effective fungicides against *A. alternata*. In present investigation also, Propiconazole, Hexaconazole and Difenoconazole was found most effective fungicide. The other effective fungicide, a

combination product Carbendazim + Mancozeb (Sixer) reported here is new information. The results of earlier workers are also in agreement with the results obtained in the present investigations.



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