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RESEARCH ARTICLE

Management of *Alternaria burnsii* causing blight of cumin

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ABSTRACT

Cumin is an important seed spice crop of India. Alternaria blight is one of the most important limiting factors for production of cumin. The studies were conducted on the management of Alternaria blight of cumin in Rajasthan. On the basis of *in vitro* studies, the isolates exhibited variable sensitivity to fungicides. Mancozeb completely inhibited the mycelial growth of the isolate Ab03, while the other isolates were less sensitive to mancozeb. Tebuconazole completely inhibited the mycelial growth of all the five isolates of *A. burnsii,* followed by azoxystorbin, carbendazim and mancozeb. Neem formulations Azadirachtin was also found effective *in vitro*. Under pot culture, combination of tebuconazole and Azadirachtin was found most effective when applied as mixed foliar spray. The application of fungicide and botanical resulted in significantly greater disease control, over their individual applications as well as over untreated control.

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INTRODUCTION

Cumin is an important seed spice crop of several sub-tropical countries. Sustainable cumin cultivation is continuously challenged by diseases that cause quantitative and qualitative losses in yield. Alternaria blight is considered the most devastating disease of cumin in sub tropical countries. It is quite prevalent and destructive as it affects all above ground plant parts including seed, thus, causing direct yield loss. Losses up to 70% have been reported (Holliday, 1980). *Alternaria burnsii* causing blight of cumin was recorded for the first time in Pakistan (Shakir *et al.*, 1995). In India blight of cumin caused by *A. burnsii* was first reported by Uppal *et al.* (1938). From Rajasthan it was first reported by Joshi (1955). The disease is considered to be a major constraint in sustainable cumin production and various control strategies include use of fungicides, biological agents, botanicals and their combinations. However, several factors including pathogenic variability influence the efficacy of these management practices. Investigations were made to devise an effective management strategy for Alternaria blight of cumin.

MATERIAL AND METHODS

Cultures of *A. burnsii* were isolated from the diseased plant samples of cumin showing variable symptoms collected from different locations and designated Ab01; Jalore, Ab02 and Ab03; (RCA and Sisarma) Udaipur, Ab04; Bikaner and Ab05; Jodhpur. The studies for management of disease were undertaken with all five isolates *in vitro* and in pot experiment, which was the most pathogenic.

***In vitro* evaluation of fungicides :**

Four fungicides *viz*., bavistin, tebuconazole, dithane M-45 and azoxtstorbin were evaluated *in vitro* against *A. burnsii* by poision food technique (Nene and Thapaliyal, 1993) at three concentrations *viz*., 250, 500 and 1000 ppm. The calculated quantities of fungicides were thoroughly mixed in the molten almost cool PDA medium before pouring into Petri-plates so as to get desired *i.e*. 250, 500 and 1000 ppm concentration of the individual fungicide. 20 ml of fungicide amended medium was poured in each 90 mm sterilized Petriplates and allowed to solidify. The plates were aseptically

inoculated with 5 mm disc cut from the periphery of 7 days old actively growing cultures of each of the five isolates of *A. burnsii* and controls without fungicides were maintained for comparison. The experiments were conducted in completely randomized design (CRD) with five replication in each treatment and the inoculated plates were incubated at 28±1^0 C. The colony diameter was measured after 7 days when the control plates were full of fungal growth. Per cent inhibition of mycelial growth was calculated by using formula given by Bliss (1934) as:

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

where,

I = Per cent inhibition

C = Colony diameter in control;

T = colony Diameter in treatment

***In vitro* evaluation of botanicals (neem formulations) :**

Efficacy of two neem based formulations *viz*., Azadirachtin (0.2%) and neem oil (0.2 %), for commercial purpose were tested against each of the five isolates of *A. burnsii* by poisoned food technique. The calculated quantities of formulations were incorporated in PDA to attain desired concentration (0.2 %) and then PDA was dispersed in sterilized 90 mm glass Petri plates for comparison from plates having PDA without neem formulations kept as control. For each treatment, five replications were taken. The plates were aseptically inoculated with 5 mm disc cut from the periphery of 7 days old actively growing cultures of each of the five isolates of *A. burnsii* and controls without botanicals were maintained for comparison. The plates were incubated at 28±1^{0}C for 7 days and then colony diameters were measured and compared with control plates where the respective pathogen was grown on PDA without neem formulations. The per cent inhibition of mycelial growth was calculated by using the formula as described earlier.

***In vivo* evaluation of fungicides and botanicals :**

The best effective fungicides found *in vitro* were assessed alone and in combination with Azadirachtin against *A. burnsii* as spray application for management of cumin blight under pot culture. The experiment was conducted in pots (30 cm) in Randomized Block Design (RBD) with three replications. The pots were filled with sand, soil and FYM (3:1:1 mixture) for the experiment. For each treatment, surface sterilized (0.1% $HgCl_2$ for 2 minutes) seeds of cumin local land race were sown. After germination, thinning was done to maintain 10 plants per pot. The experiment was conducted in pot culture in Completely Randomized Design (CRD) with three replications. 45 days old plants were spray inoculated with the conidial suspension of $1x10^4$ conidia ml^{-1} concentration. Solution of bavisitin (0.2%), diathane – M 45 (0.3%), tebuconazole (0.2%), azoxystorbin (0.2%) and Azadirachtin (0.2%) and neem oil (0.2%) alone and a combination of tebuconaole with Azadirachtin were sprayed after 36 hrs of inoculation of the most pathogenic (Ab01) isolate. For comparison, suitable inoculated control was maintained without fungicidal/ botanical application. Observations of disease severity were recorded after 10 days of inoculation on a standard disease rating scale (1-5 score) and PDI calculated. For comparison, inoculated control was maintained without fungicidal/ botanical application. Observations of disease severity were recorded after 10 days of inoculation on a standard disease rating scale (1-5 score).The per cent disease index (PDI) and per cent efficacy of disease control (PEDC) were calculated by using following formula given by Chester (1959) and Wheeler (1969) :

$$PDI = \frac{\text{Sum of all individual disease rating}}{\text{Total No. of plants ass.} \times \text{maximum rating}} \times 100$$

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

PEDC = Per cent efficacy of Disease Central

Table 1: *In vitro* effect of fungicides against *Alternaria burnsii* isolates at 250 ppm conc. after 7 days at 28±1^{0}C (poisoned food technique)

Treatments	Colony diameter(mm)*					Per cent growth inhibition**				
	Ab01	A02	Ab03	Ab04	Ab05	Ab01	Ab02	Ab03	Ab04	Ab05
Tebuconazole	0.0	0.0	0.0	0.0	0.0	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)
Azoxystorbin	35.7	38.2	28.5	32.3	80.9	60.3 (50.9)	57.5 (49.3)	68.3 (55.7)	64.0 (53.1)	10.0 (18.1)
Carbendazim	40.2	52.8	33.8	42.8	83.4	55.3 (48.0)	41.3 (39.3)	62.4 (52.1)	52.4 (4.3)	7.2 (15.0)
Mencozeb	47.3	60.4	0.0	80.1	85.2	47.4 (43.5)	32.9 (34.0)	100 (90.0)	10.9 (19.1)	5.2 (13.0)
Control	90	90	90	90	90	0.0	0.0	0.0	0.0	0.0
	SEm±		CD at 5%		CD at 1%	SEm±		CD at 5%		CD at 1%
Fungicide	0.45		1.29		1.77	0.35		0.99		1.35
Isolate	0.45		1.29		1.77	0.35		0.99		1.35
FXI	1.02		2.88		3.94	0.79		2.20		3.02

* Mean of five replications; Figures in parentheses are arcsine √ per cent angular transformed values

RESULTS AND DISCUSSION

In the present study, four fungicides at different concentrations had statistically significant effect on the mycelial growth of *A. burnsii* at all concentrations of 250, 500 and 1000 ppm, but the isolates showed variable sensitivity to the fungicides (Tables 1, 2 and 3). The effect was most pronounced on Abo3 isolate, as its growth was completely inhibited by the two fungicides tebuconazole and mencozeb at 250, 500 and 1000 ppm. On the other hand, only tebuconazole completely inhibited the growth of rest all other isolates of *A. burnsii* on all the three concentrations. Similarly, two neem based formulations *viz*., neem oil and Azadirachtin were evaluated at 0.2 per cent concentration with poison food technique against all the five isolates of *A. burnsii*. All the test botanicals significantly inhibited the mycelial growth of the five isolates of *A. burnsii*, through the isolates showed variations in sensitivity towards the two botanicals (Table 4). As well as four fungicides- tebuconazole, azoxystorbin, carbendazim and mancozeb, two botanicals, neem oil and Azadirachtin were found effective *in vitro* were further evaluated as spray applications individually and also in combination of most effective fungicide (tebuconazole) and botanical (Azadirachtin) *in vitro* studies. The lowest disease (30.4% PDI) and maximum efficacy of disease control (61.5%) was achieved by spraying a combination of tebuconazole and Azadirachtin as compared to 79.2% disease in the untreated control. This was followed by tebuconazole alone where 32.2% disease caused 59.3% PEDC. Azoxystorbin and carbendazim also showed significant suppression of the disease with (36.8% and 43.6%) PDI and (53.5% and 44.8 %) PEDC, respectively. Mencozeb spray resulted 45.8% PDI and 42.1% PEDC. However the differences in per cent disease due to mixture of tebuconazole + *Azadirachtin* was statistically non-significant over the individual application of tebuconazole. Tebuconazole was statistically (P= 0.05) significantly better effective than the other fungicides, where the difference in PDI in carbendazim and mencozeb was statistically non significant. The foliar spray with neem oil and Azadirachtin was less effective than the fungicides. Neem oil sprays @ 0.2% resulted in 54.6% PDI and 31.0% PEDC, while Azadirachtin caused 48.8% PDI and 38.2% PEDC and found effective as compared to 79.2 PDI in the untreated control. However, Azadirachtin showed statistically (P= 0.05) significantly higher disease suppression over the neem oil as well as mancozeb (Table 5).

Table 2 : *In vitro* effect of fungicides against *Alternaria burnsii* isolates at 500 ppm conc. after 7 days at 28±1^{0}C (poisoned food technique)

Treatments	Colony diameter(mm)*					Per cent growth inhibition**				
	Ab01	Ab02	Ab03	Ab04	Ab05	Ab01	Ab02	Ab03	Ab04	Ab05
Tebuconazole	0.0	0.0	0.0	0.0	0.0	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)
Azoxystorbin	29.1	26.2	18	26.8	52.8	67.7 (55.3)	70.9 (57.3)	79.9 (63.4)	70.2 (56.9)	41.3 (39.9)
Carbendazim	31.3	28.1	26.8	30.4	58.4	65.2 (53.8)	68.8 (56.0)	70.2 (56.9)	66.1 (54.4)	35.0 (36.2)
Mencozeb	36.4	33.8	0.0	78.2	59.7	59.5 (50.1)	62.4 (52.1)	100 (90.0)	12.9 (20.7)	33.5 (35.3)
Control	90	90	90	90	90	0.0	0.0	0.0	0.0	0.0
	SEm±		CD at 5%		CD at 1%	SEm±		CD at 5%		CD at 1%
Fungicide	0.40		1.13		1.55	0.28		0.79		1.07
Isolate	0.40		1.13		1.55	0.28		0.79		1.07
F X C	0.90		2.53		3.47	0.62		1.75		2.40

* Mean of five replications; Figures in parentheses are arcsine √ per cent angular transformed values

Table 3 : *In vitro* effect of fungicides against *Alternaria burnsii* isolates at 1000 ppm conc. after 7 days at 28±1^{0}C (poisoned food technique)

Treatments	Colony diameter(mm)*					Per cent growth inhibition**				
	Abo1	Ab02	Ab03	Abo4	Abo5	Ab01	Ab02	Ab03	Ab04	Ab05
Tebuconazole	0.0	0.0	0.0	0.0	0.0	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)	100 (90.0)
Azoxystorbin	23.5	11.2	6.6	6.5	26.8	73.9 (59.2)	87.5 (69.3)	92.6 (74.2)	92.7 (74.4)	70.1 (56.9)
Carbendazim	28.7	12.8	14.2	22.6	28.3	68.1 (55.6)	85.8 (67.8)	84.1 (66.5)	74.8 (59.9)	68.5 (55.8)
Mencozeb	34.6	15.6	0.0	59.7	29.1	61.5 (51.6)	82.7 (65.3)	100 (90.0)	33.5 (35.3)	67.6 (55.3)
Control	90	90	90	90	90	0.0	0.0	0.0	0.0	0.0
	SEm±		CD at 5%		CD at 1%	SEm±		CD at 5%		CD at 1%
Fungicide	0.31		0.90		1.22	0.19		0.51		0.71
Isolate	0.31		0.90		1.22	0.19		0.51		0.71
FXI	0.71		2.00		2.74	0.41		1.15		1.58

* Mean of five replications; Figures in parentheses are arcsine √ per cent angular transformed values

In a similar study, growth and sporulation of *A. burnsii* at different concentrations of various systemic and non-systemic fungicides were studied by Patel (1993). Chand *et al*. (2000) evaluated efficacy of various fungicides on inoculated healthy seeds of cumin with *Alternaria* spp. Among them, mancozeb resulted highest seed germination (94%) and minimum pre and post-germination mortality (2.0 and 1.5%) followed by others. In another studies also mancozeb completely inhibited (94.09%) the growth at half of the recommended dose, The study indicated that all the isolates were sensitive to mancozeb (Pipaliya and Jadeja, 2008 and Vihol *et al*., 2009).

The results are in agreement with several workers. Jadeja and Pipliya (2008) reported that 5% and 10% extract of garlic cloves and ginger rhizomes were most effective resulting in 78.5% and 73% mean inhibition of *A. burnsii*. Vihol *et al.* (2009) reported that the extract of garlic bulb (*Allium sativum*) at 15% inhibited the mycelial growth (67%) of *A. burnsii in vitro*.

However, Lakhtaria and Pillai (1978) evaluated fungicide against blight of cumin through seed treatment plus spraying with bavistin (carbendazim) which gave very good control of *A. burnsii*. Mehta and Solanki (1990) recommended four sprays of Dithane M-45 (0.2%) for the effective control of the disease. Azoxystrobin proved to be effective against Alternaria blight of *raya* and carrot under field condition (Chander *et al.*, 2005 and Stanislaw and Nawrocki, 2007).

These results are also in agreement with findings of Gangopadhyay *et al.* (2010) who observed the effects of five plant extracts (botanicals) *viz.*, *Aloe vera*, *Calotropis procera*, *Eucalyptus golobulus*, *Azardiratcha indica* leaves and *A. indica* seed kernel on growth and spore germination of *A. burnsii*. The study showed that at 10% concentration, the inhibition was most effective (*in vitro* and *in vivo*). Polra and Jadeja (2011) evaluated 12 fungicides and 6 phytoextracts against *A. burnsii*. In laboratory screening, systemic fungicides, hexaconazole and tebuconazole and non-systemic fungicide, mancozeb proved most effective. Garlic clove and ginger rhizome extract proved to be the best sources of plant origin for radial growth and spore germination inhibition of pathogen. On pot grown plants inoculated with *A. burnsii*, the combination of tebuconazole (0.1%) + Azadirachtin (0.2%), followed by tebuconazole alone was found the most effective to manage Alternaria blight of cumin. The spray application of the fungicides and botanical in integration resulted in

Table 4: *In vitro* effect of botanicals against *Alternaria burnsii* isolates at 0.2 % conc. after 7 days at 28±1^{0}C (poisoned food technique)

Treatments	Colony diameter(mm)*					Per cent growth inhibition**				
	Ab01	Ab02	Ab03	Ab04	Ab05	Ab01	Ab02	Ab03	Ab04	Ab05
Azadirachtin	54.2	69.8	63.3	60.2	61.8	39.7 (39.0)	22.3 (28.0)	29.5 (32.8)	32.9 (35.0)	29.5 (33.9)
Neem oil	70.9	75.8	74.6	72.4	76	21.2 (27.3)	16.3 (23.6)	17.0 (24.1)	19.4 (26.0)	15.4 (22.8)
Control	90	90	90	90	90	0.0	0.0	0.0	0.0	0.0
	SEm±		CD at 5%		CD at 1%	SEm±		CD at 5%		CD at 1%
Botanicals	0.68		1.91		2.59	0.60		1.68		2.95
Isolate	0.87		2.46		3.34	0.77		2.17		2.95
FXI	1.51		4.26		5.79	1.33		3.77		5.12

* Mean of five replications; Figures in parentheses are arcsine √ per cent angular transformed values

Table 5 : Relative efficacy of foliar spray of promising fungicides / botanicals / fungicides +botanicals on Alternaria blight of pot grown plants of cumin

Sr. No.	Fungicides / botanicals with concentration	Percent disease index (PDI) *	(PEDC)**
1.	Tebuconazole	32.2 (34.5)	59.3 (50.3)
2.	Azoxystorbin	36.8 (37.3)	53.5 (47.0)
3.	Carbendazim	43.6 (41.3)	44.8 (42.0)
4.	Mancozeb	45.8 (42.5)	42.1 (40.4)
5.	Azadirachtin	48.8 (44.3)	38.2 (38.1)
6.	Neem oil	54.6 (47.6)	31.0 (33.8)
7.	Tebuconazole + *Azadirachtin*	30.4 (33.4)	61.5 (51.6)
8.	Control	79.2 (62.9)	–
SEm±		0.69	0.76
CD (P=0.05)		2.07	2.27

*Mean of three replications; Figures in parentheses are arcsine √ per cent angular values;
** Per cent efficacy of disease control

significant increase in efficacy of disease control over their individual applications over the inoculated untreated control. This treatment seems quite feasible and economical and can be recommended for management of Alternaria blight in fields.

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