

Management of *Alternaria* blight caused by *Alternaria alternata* in *Adusa* (*Adhatoda vasica*)

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Received: 4 November 2009; Accepted: 11 May 2010

ABSTRACT

The efficacy of 3 fungicides and 5 phyto-extracts against *A. alternata* were evaluated *in vitro* and *in vivo* in *Adusa* (*Adhatoda vasica* Nees). Mancozeb was found to be the most effective fungicide in checking the mycelial growth and conidial germination of *A. alternata*. It also gave maximum (64.60%) disease control when sprayed twice (@ 0.25%) on artificially inoculated *Adusa* plants. Among the 5 phyto-extracts tested, garlic (*Allium sativum* L.) clove-extract proved as the most effective treatment in checking growth and conidial germination of the pathogen and also in controlling the leaf blight. Two sprays of garlic extracts (@ 15%) gave 55.12% disease control on artificially inoculated *A. vasica* plants.

Key words: *Adhatoda vasica*, *Alternaria alternata*, Blight, Fungicide, Phyto extract

Adhatoda vasica (syn. *Justicia adhatoda*), also known as malabar nut or *Adusa* is an important medicinal plant in traditional as well as modern systems of medicines. It grows abundantly in wastelands, particularly on foothills and rocky places. The leaves are used, either alone or in combination with other drugs, for preparation of expectorants (Jain and DeFilipps 1991, Singh *et al.* 1996). Its roots, leaves and flowers yield an oil that is effective against *Tubercle bacilli* (Singh *et al.* 1996). The drug *Vasaka* is prepared from the fresh or dried leaves of the plant. The blight caused by *A. alternata* affects foliage and inflorescence of the plant (Fig 1). Infection of leaves makes approximately one-fifth foliage of the *A. vasica* plant unfit for medicinal use (Singh *et al.* 2006). Search of literature revealed that so far no work has been carried out on management of the disease. Therefore, keeping in view the medicinal importance of the plant, particularly its leaves, the study was conducted on management of the disease through fungicides vis-à-vis some eco-friendly products like phyto-extracts.

MATERIALS AND METHODS

Efficacy of fungicides, viz mancozeb (75WP), copperoxychloride (50WP), carbendazim (50WP) and extracts

* Based on a part of Ph D thesis of the first author submitted to the Rajasthan Agricultural University, Bikaner during 2005

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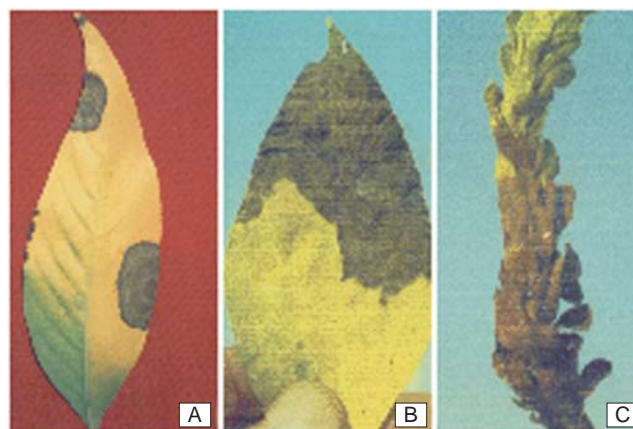


Fig 1 Symptoms of *Alternaria* blight on leaves (AB) and inflorescence (C) of *Adhatoda vasica*

of garlic (*Allium sativum* L.) clove, onion (*Allium cepa* L.) bulb, neem (*Azadirachta indica* A. Juss) leaves, periwinkle [*Catharanthus roseus* (L.) Reihb] leaves and sacred basil (*Ocimum sanctum* L.) (*tulsi*) leaves were tested against *A. alternata* both *in vitro* and *in vivo*.

Efficacy of each fungicide against mycelial growth and conidial germination of *A. alternata* was tested at 3 concentrations, viz 50, 100 and 150 ppm. The phyto-extracts were tested at 5, 10 and 15% concentrations. For this, the required plant part was thoroughly washed with sterilized distilled water and were grinded separately in electric grinder using equal amount of sterilized distilled water to get stock solution. The mixture was squeezed through double layered sterilized cheese cloth. The extract thus obtained was

considered as of 100% concentration. It was further diluted to get 5, 10 and 15% of concentrations using sterilized distilled water. Their effect against colony growth was tested using poisoned-food-technique. Required quantity of fungicide or phyto-extracts were added separately in sterilized and melted potato dextrose agar medium aseptically under laminar flow and thoroughly mixed to get desired concentration. Amended medium was poured in sterilized Petri-dishes under laminar flow and were allowed to solidify for 12 hr. Each plate was then inoculated with 2 mm disc of mycelial bit taken from the periphery of 7-day colony of *A. alternata* growing on potato dextrose agar medium. The inoculated Petri-dishes were incubated at $25 \pm 1^\circ\text{C}$ in biological oxygen demand incubator. Four plates were used for each treatment serving as 4 replications. Colony diameter (2 diagonals) was measured after 6 days of incubation. Medium without fungicide/ phyto-extract served as control. Per cent growth inhibition was calculated by Vincent's formula (1947).

For spore germination assay, the spore suspension was prepared in sterilized distilled water to get 10 to 12 conidia/microscopic field ($10\times$). A loop full of spore suspension was put on a clean glass slide and allowed to air-dry. Just immediately after air-drying, a drop of fungicidal/ phyto-extract suspension of required concentration was put separately on the air-dried surface on glass slide. The slide was then accommodated in a moist chamber made by placing 2 moist filter papers in the bottom of a Petri-dish. Two glass rods were positioned on the bottom of each Petri-dish over the filter papers to support the glass slide. Two slides were accommodated in each moist chamber and incubated for 24 hr at $25 \pm 1^\circ\text{C}$. The germination of spores was recorded after 24 hr of incubation by examining 8 slides constituting 4 replications of a treatment. Before microscopic examination, a drop of lactophenol was added. Sterilized water served as control.

Efficacy of fungicides and phyto-extracts was tested under cage house conditions. About 8-month-old *A. vasica* plants raised in cage house were inoculated by spraying spore suspension of *A. alternata* (1×10^3) with an atomizer. The check was maintained by spraying the sterilized water only. Treated and control plants were covered with polyethylene bags to provide identical condition. The first spray of fungicides/phyto extracts was applied separately after day 7 of inoculation, when disease symptoms were initiated on the inoculated foliage. The spray was repeated after 15 days interval. Fungicides, viz mancozeb and copperoxychloride were used at 3 concentrations, i.e. 0.15, 0.20 and 0.25% whereas carbendazim was applied at 0.05, 0.10 and 0.15% concentrations. The phyto-extracts were used at 3 concentrations, i.e. 5, 10 and 15%. Three plants were sprayed with each concentration to serve as 3 replications. Observations of disease intensity were recorded one month after spray. For this, 25 leaves randomly taken from each plant were assessed for per cent leaf area affected with the help of disease assessment key (Singh 2005) as healthy; 1, up to 10; 2, > 10 to 25; 3, > 25 to 50; 4, > 50 to 75 and 5, > 75% leaf area infected. From this, per cent disease intensity was computed. From the mean per cent disease intensity (PDI), per cent disease control (PDC) was calculated using formula given by Wheeler (1969). Plants sprayed with water served as control.

RESULTS AND DISCUSSION

Efficacy of fungicides against mycelial growth

Data presented in Table 1 show that all the 3 fungicides caused significant reduction in mycelial growth as compared to check. Mancozeb was most effective, followed by copperoxychloride and carbendazim, resulting in significant reduction of mycelial growth of *A. alternata*. Mancozeb at 150 ppm gave maximum growth inhibition (89.52%), whereas minimum inhibition (18.15%) was recorded in

Table 1 *In vitro* efficacy of fungicides against mycelial growth and conidial germination of *A. alternata* after incubation at $25 \pm 1^\circ\text{C}$ for 6 days and 24 hr, respectively

Fungicide	Per cent inhibition of mycelial growth* concentration in ppm			Mean	Per cent inhibition of conidial germination* concentration in ppm			Mean
	50	100	150		50	100	150	
Mancozeb	58.87(49.64)	75.28(60.01)	89.52(70.63)	74.55(59.33)	68.23(55.61)	83.20(65.61)	91.30(72.53)	80.91(63.45)
Copperoxychloride	42.03(40.40)	53.14(46.70)	68.87(55.64)	54.68(47.33)	53.34(46.71)	62.67(51.93)	71.32(57.41)	62.44(51.92)
Carbendazim	18.15(25.10)	39.07(38.60)	59.52(50.23)	38.91(38.15)	30.67(33.23)	43.34(41.01)	46.84(42.74)	40.28(39.21)
Check	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
Mean	29.76(32.64)	41.87(39.84)	54.47(47.32)	42.03(40.40)	38.06(38.06)	47.30(43.28)	52.36(46.15)	45.90(42.19)
	SEm \pm CD ($P=0.05$)				SEm \pm CD ($P=0.05$)			
Fungicides (F)	0.49	1.39			0.55	1.57		
Concentration (C)	0.42	1.20			0.48	1.36		
F \times C	0.85	2.40			0.96	2.73		

* Average of 4 replications each

Figures in parentheses are angular transformed values

Table 2 Efficacy of fungicides in controlling *Alternaria* blight of *Adusa* in cage house conditions

Fungicide	Concentration (%)	Per cent disease intensity*	Per cent disease control
Mancozeb	0.15	26.73(30.74)	55.70(47.94)
	0.20	23.57(28.73)	60.93(50.85)
	0.25	21.36(27.31)	64.60(53.13)
Copperoxychloride	0.15	36.81(36.94)	38.99(38.15)
	0.20	32.53(34.43)	46.08(42.70)
	0.25	30.11(33.20)	50.09(45.00)
Carbendazim	0.05	41.25(39.81)	31.63(33.83)
	0.10	36.54(36.93)	39.44(38.32)
	0.15	31.42(33.82)	47.52(43.35)
Check (water spray)		60.34(50.81)	0.00(0.00)
	SEm±	CD (P=0.05)	
Fungicides (F)	0.43	1.23	
Concentration (C)	0.37	1.06	
F × C	0.75	2.13	

* Average of 3 replications each

Figures in parentheses are angular transformed values

carbendazim 50 ppm. Fungicides × concentration interactions were also significant.

Efficacy of fungicides against conidial germination

All the 3 fungicides caused significant reduction in

conidial germination (Table 1). Mancozeb gave maximum inhibition of conidial germination (80.91%), followed by copperoxychloride (62.44%) and carbendazim (40.28%). Fungicide × concentration interactions were also significant, indicating that efficacy of fungicides increases with increasing concentration.

Efficacy of fungicides in controlling blight of *A. vasica*

The result presented in Table 2 revealed that all the fungicides at all the 3 concentrations were found significantly superior over check in controlling the disease. Maximum disease control (64.60%) was recorded by mancozeb (0.25%) when sprayed twice. It was followed by copperoxychloride (0.25%) and carbendazim (0.15%). Increasing concentration caused significant increase in efficacy of all the fungicides which indicates that fungicides × concentration interactions are significant. Mancozeb being a broad spectrum fungicide has been reported effective against number of *Alternaria* diseases of various crops, like sunflower (Rao and Ranganatha 2003), Frenchbean (Singh *et al.* 2003) and brinjal (Singh and Rai 2003).

Efficacy of phyto-extracts against mycelial growth

Garlic clove extract was found most efficacious in checking mycelial growth of *A. alternata* (Table 3). It gave 55.41% inhibition, followed by *neem* (52.29%), periwinkle (46.94%), *tulsi* (42.33%) and onion (35.86%) extracts.

Table 3 *In vitro* efficacy of phyto-extracts against mycelial growth and conidial germination of *A. alternata* after incubation at 25 ± 1°C for 6 days and 24 hr, respectively

Phyto-extract	Plant part used	Per cent inhibition of mycelial growth* concentration (%)			Mean	Per cent inhibition of conidial germination* concentration (%)			Mean
		5	10	15		5	10	15	
Garlic	Clove	42.17	55.63	68.44	55.41	63.57	74.15	82.57	73.13
		(40.46)	(47.93)	(55.62)	(47.92)	(52.53)	(59.30)	(64.93)	(58.72)
Onion	Bulb	31.15	36.07	40.37	35.86	52.62	57.35	63.58	57.85
		(33.80)	(36.90)	(39.21)	(36.34)	(46.13)	(49.01)	(52.53)	(49.04)
<i>Tulsi</i>	Leaves	34.12	43.33	49.56	42.33	58.30	62.57	69.32	63.39
		(33.76)	(41.01)	(44.43)	(40.41)	(49.61)	(51.93)	(56.21)	(52.51)
<i>Neem</i>	Leaves	39.75	52.48	64.65	52.29	60.00	66.32	78.53	68.28
		(38.64)	(46.12)	(53.13)	(46.11)	(50.80)	(54.31)	(62.03)	(55.61)
Periwinkle	Leaves	36.33	48.00	56.50	46.94	56.52	64.32	71.00	63.94
		(36.91)	(43.90)	(48.43)	(42.75)	(48.43)	(53.11)	(57.40)	(52.55)
Check	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Mean		30.58	39.25	46.58	38.80	48.50	54.12	60.83	54.43
		(33.23)	(38.61)	(42.73)	(38.14)	(43.85)	(47.29)	(50.77)	(44.35)
		SEm±	CD (P=0.05)			SEm±	CD (P=0.05)		
Extract (E)		0.37	1.06			0.42	1.20		
Concentration (C)		0.26	0.75			0.29	0.84		
E × C		0.64	1.84			0.73	2.08		

* Average of 4 replications each

Figures in parentheses are angular transformed values

Table 4 Efficacy of phyto-extracts in controlling *Alternaria* blight of *Adusa* in cage house conditions

Phyto-extract	Plant part use	Concentration (%)	Per cent disease intensity*	Per cent disease control
Garlic	Clove	5	38.53(38.13)	38.75(38.14)
		10	33.18(35.10)	47.25(43.31)
		15	28.23(31.91)	55.12(47.90)
Onion	Bulb	5	52.73(46.14)	16.18(23.60)
		10	48.70(43.94)	22.58(28.03)
		15	44.62(41.63)	29.07(32.60)
<i>Tulsi</i>	Leaves	5	48.85(43.94)	22.34(28.01)
		10	43.50(41.03)	30.85(33.24)
		15	39.57(38.63)	37.10(37.50)
<i>Neem</i>	Leaves	5	42.57(40.43)	32.33(34.41)
		10	37.73(36.94)	40.02(39.20)
		15	31.75(33.80)	49.53(44.43)
Periwinkle	Leaves	5	44.17(41.60)	29.78(32.64)
		10	40.27(39.21)	35.98(36.35)
		15	34.82(35.74)	44.65(41.63)
Check (water spray)			62.91(51.95)	0.00(0.00)
			SEm±	CD (P=0.05)
		Extract (E)	0.48	1.36
		Concentration (C)	0.34	0.96
		E × C	0.83	2.36

* Average of 3 replications each

Figures in parentheses are angular transformed values

Extract × concentration interactions were also significant.

Efficacy of phyto-extracts against conidial germination

Garlic extract was significantly superior in checking conidial germination over all other extracts and the control. It gave 73.13% inhibition of conidial germination (Table 3). It was followed by *neem* (68.28%), periwinkle (63.94%), *tulsi* (63.39%) and onion (57.85%). Extract × concentration interactions were also significant.

Efficacy of phyto-extracts in controlling blight of *A. vasica*

Garlic clove extract (15%) gave maximum disease control (55.12%). It showed 28.23% disease intensity as against 62.91% in check (Table 4). All other phyto-extracts tested were also found significantly effective over check. Onion bulb extracts was least effective. Extract × concentration interactions were also significant. Earlier workers have also found garlic clove extract to be effective against *Alternaria* spp *in vitro* and *in vivo* pathogenic to various crops like potato (Chaudhary *et al.* 2003), tomato (Prasad and Naik 2003) and sunflower (Amaresh and Nargund 2003). The antifungal properties of the plant extract may be due to their antimicrobial substance present in the extract. Cavallito *et al.* (1974) reported that *A. sativum* contains an important allyl compound, viz Allium. (diallyl disulphide). The active compounds reported in *A. sativum* are allisatin I, allisatin II, garlic and garliphytocide etc. (Sharma and Prasad 1980).

The results obtained on control of *Alternaria* blight of *Adusa* in the present study are encouraging both with respect

to fungicides and plant extracts. Since *Adusa* is a perennial plant and the pathogen survives in infected plant tissues, use of chemicals for control of the disease should be done with caution keeping in view that any chemical applied should not last longer in/on the leaves and also should not interfere with the medicinal properties of the leaves. It is therefore likely that botanicals (plant extracts) may do better than fungicides for such plants. Plant extracts may be used as such in crude form or their active principal(s) may be isolated and along with suitable inert material be used as dust/spray on crops. Until these eco-friendly formulations (dusts/sprays) are made available for use at field scale, the fungicides may only serve as stop-gap arrangement and should not find a permanent place at least in production technology of medicinal plants.

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