

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; 9(1): 118-122
www.biochemjournal.com
Received: 06-11-2024
Accepted: 10-12-2024

Megha Mandloi
Department of Plant
Pathology, B.M. College of
Agriculture, Khandwa,
Madhya Pradesh, India

SK Arsia
Department of Plant
Pathology, B.M. College of
Agriculture, Khandwa,
Madhya Pradesh, India

MK Kureel
Department of Horticulture,
B.M. College of Agriculture,
Khandwa, Madhya Pradesh,
India

OP Bharti
Department of Plant
Pathology, Krishi Vigyan
Kendra, JNKVV, Jabalpur,
Madhya Pradesh, India

Satish Sharma
Department of Plant
Pathology, B.M. College of
Agriculture, Khandwa,
Madhya Pradesh, India

Kuldeep Gupta
Department of Plant
Pathology, B.M. College of
Agriculture, Khandwa,
Madhya Pradesh, India

Corresponding Author:
Megha Mandloi
Department of Plant
Pathology, B.M. College of
Agriculture, Khandwa,
Madhya Pradesh, India

Organic management of Alternaria leaf spot of cotton

Megha Mandloi, SK Arsia, MK Kureel, OP Bharti, Satish Sharma, and
Kuldeep Gupta

DOI: <https://doi.org/10.33545/26174693.2025.v9.i1b.3464>

Abstract

Cotton is the most important fiber in the world. It belongs to the botanical family "Malvaceae". Cotton is referred to as "king of Fibres" and is also popularly known as "white Gold". Alternaria leaf spot of cotton is the major disease caused due to invade of *Alternaria alternata*. Three cow products and three botanicals viz., Cow dung, Cow urine, Milk whey, *Azadiracta indica* (leaf) extract, Garlic bulb extract and *Calotropis gigantean* (leaf) extract were incorporated to evaluate against *Alternaria alternata*. The treatments were used at 10%, 20% and 25% concentrations in vitro conditions during the experiments. Maximum inhibition of mycelium was observed in *Azadiracta indica* (leaf) extract is 42.24% followed by Garlic bulb extract (39.77%) and Cow urine (37.37%) while minimum inhibition of mycelium in Cow dung (25.86%) followed by Milk whey (28.61%) and *Calotropis gigantean* (leaf) extract 50% WP (33.35%). On the basis of colony colour, *Alternaria alternata* pathogen treated with different cow products and botanicals against Alternaria leaf spot of cotton was observed Greyish black colony colour in all treatments except cow urine and milk whey which shows Greyish colony colour after inoculation under *in vitro* condition. Under *in-vivo* conditions, Maximum disease reduction (%) was recorded in *Azadiracta indica* (leaf) extract is 32.52% followed by Garlic bulb extract is 29.27% and Cow urine is 26.84% while minimum disease reduction (%) was recorded in cow dung is 17.08% followed by Milk whey is 21.95% and *Calotropis gigantean* (leaf) extract is 23.58%.

Keywords: Cotton, *Alternaria alternata*, *Alternaria leaf spot*, botanicals, cow products

Introduction

Cotton is one of the most important fiber crops of India and plays a dominant role in the agricultural economy of India. It provides the basic raw material as cotton fibre to cotton textile industry. In India, cotton provides direct livelihood to 60 lakh farmers and about 4 -5 crore people are employed in cotton trade and its processing. Cotton is referred to as "king of Fibres" and is also popularly known as "white Gold". India is the largest cotton growing country in the world with an area of around 12.24 MH. Total production of cotton in India is 36.1 million bales of 170 kg and productivity is 501 kg ha⁻¹. India's share in global cotton exports is around 22% (Anonymous, 2019) [1]. Cotton has major share in global agriculture economy; however, its production and yield are compromised due to various biotic, abiotic, and climatic factors. To meet the global need, innovative research has been carried out to enhance the cotton production. Starting from the conventional breeding strategies to the modern technologies, there is a long history of cotton research (Zaib *et al.*, 2020) [14]. Alternaria leaf spot is generally associated with late-season development of cotton plants or senescing plant tissues. It is characterized by small, brown, circular lesions on the leaves that are surrounded by distinct purple margins. Similar symptoms can be observed on buds, flowers, and bolls in mature plants. As the disease progresses, the lesions expand and become dry and gray in the centre, exhibiting shot-hole symptoms on leaves. These spots may coalesce and occupy large leaf areas resulting in severe defoliation, affecting cotton yield and quality (Watkins, 1981) [13].

Materials and Methods

The present investigation was undertaken for the "Organic management strategy for Alternaria leaf spot of cotton". The investigation was carried out to test the efficacy of organic amendments under laboratory and field conditions in the Department of Plant Pathology, Bhagwantrao Mandloi College of Agriculture Khandwa, under RVSKVV,

Gwalior (M.P.). The details of materials used, experimental procedures followed and techniques adopted are given as under

The experiment was conducted at Research field of B. M. College of Agriculture, Khandwa and *In-vitro* experiments were conducted in laboratory at Department of Plant Pathology, B. M. College of Agriculture, Khandwa under RVSKVV, Gwalior (M.P.).

Isolates of *Alternaria alternata* were isolated from wilt infected plant collected from Khandwa district of M.P and they were cultured and used during the course of study. The following Organic amendments were used in *In-vitro* and *In-vivo* against *Alternaria alternata* in the Table-1. Peeled potatoes were cut into small pieces and boiled in 1/2 litre distilled water in a sauce pan for 10 min. In another pan agar powder was boiled in half litre of distilled water separately. Potato extract was filtered with the help of muslin cloth, then added to dissolve agar solution along with dextrose and heated for 5 minutes. The media was poured into flask of graduated marks of 250 ml or 500 ml. The PDA media was autoclaved by putting cotton plug on the rim of flask and covered with aluminium sheet paper at 121.6 °C for 15 minutes. Before pouring the media to petri plates, bactericide (*Streptomycin*) is added at the rate of 1.0 g/lit.

Table 1: List of Organic amendments used in experiment

S. No.	Organic amendments
1	<i>Azadiracta indica</i> (leaf) extract
2	Garlic bulb extract
3	Cow dung
4	Cow urine
5	Milk whey
6	<i>Calotropis gigantean</i> (leaf) extract
7	Control

In-vitro* evaluation of organic amendments against *Alternaria alternata

The bioassay studies were carried out by employing the Poisoned Food Technique as reported earlier. Three cow products and three botanicals viz., Cow dung, Cow urine, Milk whey, *Azadiracta indica* (leaf) extract, Garlic bulb extract and *Calotropis gigantean* (leaf) extract were incorporated aseptically in sterilized Potato Dextrose Agar medium in their original 20% concentrations.

20 ml of the Potato Dextrose Agar medium was poured in each of the sterilized petri plate. Then these petri-plates were inoculated with a 5 mm disc cut placed with a sterilized cork borer from an actively growing ten days old culture of *Alternaria alternata*.

The inoculated petri plates were incubated at 25±1 °C. Each treatment was replicated three times. Suitable control (without treatment) was also maintained. The radial growth of *Alternaria alternata* was recorded after seven and ten days. Sporulation from the pathogen was also be noted as (+) for positive sporulation and (-) for no sporulation. After recorded radial growth, we calculate the growth inhibition percentage with the following formula–

$$\text{Growth Inhibition Percent} = \frac{C - T}{C} \times 100$$

Where

C = Growth of test fungus in control

T = Growth of test fungus in treatment in mm

Observations: Percent Disease intensity (PDI), and Yield PDI will be taken from five plants of each place randomly of middle, lower and upper leaves of a plant. Five randomly selected plants in each plot and were tagged for taking observations for disease component. Percent disease intensity will be calculated as per 0 to 4 disease scale developed by Sheo Raj (1988) ^[10] table-2.

Table 2: Disease Rating and Disease reaction used in experiment

Rating scale	Per cent leaf area covered	Disease reaction
0	0	Immune (I)
1	0-10	Resistant (R)
2	11-20	Moderately resistant (MR)
3	21-40 Moderately	susceptible (MS)
4	More than 40	Susceptible (S)

In-vivo* evaluation of Organic amendments against *Alternaria alternata

An experiment was laid out in the field conditions during (2021) at Research cotton farm of B.M. College of Agriculture, Khandwa campus to manage *Alternaria* leaf spot of cotton by using botanicals extracts and cow based products and the experiment was maintained in three replications. The spray was done at fortnightly intervals. All the spray was done in 20% concentrations.

Experimental details

Design: Randomized Block Design (RBD)	Crop and variety: Cotton, JK-4
Plot size: 3.6 m x 2.7 m	Date of sowing: 10/07/2021
Spacing: 60 cm x 45 cm	Fertilizer dose: 80:40:40 NPK kg/ha
Treatments : 7	Replications: 3

All other cultural and pest management practices were imposed as recommended in package of practices. The first spray of each treatment was initiated as soon as the first symptom of leaf spot was seen in the field and repeated at an interval of 15 days. Five plants were selected randomly in each plot and observation on severity of the disease on the foliage (using 0-4 scale) was recorded under different treatments one day before each spray and 15 days after the final spray. The cotton yield in each treatment was recorded.

Results and Discussion

Alternaria leaf spot of cotton is the major disease caused due to invade of *Alternaria alternata*. It is disastrous and reduces maximum yield in major cotton growing areas of Madhya Pradesh, Maharashtra and some other states. To ascertain the problems, *in-vitro* and *in-vivo* evaluation of cow products and botanicals against pathogens has been carried out for the research purposes in present investigation “Organic management strategy for *Alternaria* leaf spot of cotton “.

Symptomology

The symptoms of *Alternaria* leaf spot of cotton was observed on different plant parts. The symptoms first appear on the upper surface of leaves in the form of dot shape, light brown to blackish in colour, circular or irregular lesions, which later on turns dark brown due to the appearance of spore masses. These spots enlarged and coalesced forming large sized spots. In the centre of such spots, light brown dot surrounded by the number of dark alternating concentric rings also appeared in some cases. Affected leaves become

dry, cracking and fall off. Veins of leaves also affected. The disease also causes cankers on the stem. The finding of Watkins (1981) ^[13] is in agreement with the present findings and he described the phenotypic symptoms of *Alternaria* leaf spot on different species of cotton were evaluated. Small, dull to dark brown, circular or irregular shaped spots appear on leaf varying in diameter from 0.5 to 10 mm. They often develop concentric rings and represent a target board like appearance which is better defined on the upper surface. When mature they have dry, grey centres which may crack and even drop. The spots may coalesce and occupy large area of the leaf

Isolation of pathogen

The investigation was carried out and observations were made for appearance of the *Alternaria* leaf spot of cotton. Symptoms on cotton plants from the trial field, collect infected leaves in polythene bags, carried to the laboratory and isolation on Potato Dextrose Agar (PDA) medium. The pathogen was isolated from infected leaves of diseased plants were taken and cut into 3 to 5 mm long pieces with some healthy portion. Infected parts sterilized with 0.1% mercuric chloride for 25 to 30 second and then washed with autoclaved distilled water for three times then aseptically plated. Isolation done in sterilized Petri plates under Laminar-Air-flow cabinet and these plates incubated in BOD incubator at 25±2 °C. 7 days after incubation, when the well-developed mycelia growth of the pathogen was seen on the PDA medium, it was aseptically transferred onto another PDA media plate.

In vitro evaluation of cow products and botanicals against *Alternaria alternata*

To evaluate different cow products and botanicals against pathogen (*Alternaria alternata*), an experiment conducted at 10%, 20% and 25% concentrations in which three cow products (Cow dung, Cow urine and Milk whey) and three botanicals (*Azadirachta indica* (leaf) extract, Garlic bulb extract and *Calotropis gigantea* (leaf) extract) respectively are used. The effect of cow products and botanicals against pathogen is analyzed by Poison food technique. The effect of various cow products and botanicals on the Colony diameter, colour, type of colony, inhibition of mycelium and Spore germination of the pathogen was observed.

Table-3 shows that all cow products and botanicals are effective in controlling growth of *Alternaria alternata* after inoculation of pathogen. Control plot showed 74.52 mm growth whereas the average growth is 17.98mm found. The minimum mean Colony diameter of 43.04 was observed in *Azadirachta indica* (leaf) extract followed by Garlic bulb extract (44.88 mm) and Cow urine (46.67 mm) whereas, the maximum Colony diameter of 55.25 mm was observed in Cow dung followed by Milk whey (53.20 mm) and *Calotropis gigantea* (leaf) extract (49.67 mm).

All these findings are in agreement with the findings of Panchal and Patil (2009) ^[5] reported that garlic clove extract (10%) was proved to be best in inhibiting mycelia growth and sporulation of *Alternaria alternata* incident of fruit rot of tomato followed by turmeric and neem leaf extract on the contrary in present investigation Neem was recorded mean inhibition of (56.66). To circumvent pollution hazards due to injudicious use of agrochemicals and also to avoid development of resistance in pathogenic fungi against commonly used fungicides, use of plant extracts for the

management of plant disease increased in recent years and proved to be very effective against plant diseases. Hassanein *et al.*, (2010) ^[2] also reported that Inhibition effect of *Azadirachta indica* was tested *Alternaria*, their inhibitory effect studied at various concentrations (5, 10 and 20%). Neem extract inhibited mycelial growth of fungi and the degree of suppression proportionally increased with increasing concentration of extracts. Maximum percent inhibition was of 20% aqueous Neem leaf extract that decreased the incidence of disease from 53.2 to 42.5% after period of 2 weeks and 100 to 79.2% after period of 4 weeks.

Colony colour and type of colony

On the basis of colony colour, *Alternaria alternata* pathogen treated with different cow products and botanicals against *Alternaria* leaf spot of cotton was observed Greyish black colony colour in all treatments except cow urine and milk whey which shows Greyish colony colour after inoculation under *in vitro* condition.

The present results of investigations are in accordance with the findings of Srinivasan (1994) ^[11] who described that *A. alternata* produces initially white colored mycelium, later at maturity it becomes dark colored in culture plates. Conidiophores and conidia are golden brown. Conidiophore is simple, straight or curved, 1-3 septate, 50x3-6 µ with one to several apical scars. Conidia often in long branched chains, ovoid, obclavate, obpyriform, rarely ellipsoidal, with or without a short, conical or cylindrical apical beak not exceeding one third the length of conidium, smooth walled or warted, singly constricted at 3-8 transverse septa, with one or two longitudinal septa at the lower part, 18-63x7-18 µ.

Inhibition of mycelium (%)

To evaluate Inhibition of mycelium cow products and botanicals against *Alternaria alternata* under *In-vitro* condition. Food poison technique was used after 7 days of inoculation and their observed growth inhibition of mycelium per cent is shown below-

The data of table 04 shows that all treatments were found slightly better over control. Two botanicals and one cow product found effective out of three cow product and three botanicals. Maximum Inhibition of mycelium was observed in *Azadirachta indica* (leaf) extract is 42.24% followed by Garlic bulb extract (39.77%) and Cow urine (37.37%) while minimum Inhibition of mycelium in Cow dung (25.86%) followed by Milk whey (28.61%) and *Calotropis gigantea* (leaf) extract 50% WP (33.35%). The findings of present investigations are similar to Turkusay and Onogur (1998) ^[12] reported that spore germination of *A. alternata* was inhibited by *Hedera helix* Lowc. extracts followed by *Datura stramonium* L. extracts. *Ficus carica* L. and *Avena sativa* L. extract also affected colony development. Karade and Sawant (1999) ^[4] tested different plant extracts on solid and liquid media against *A. alternata* and reported that *Allium sativum* L. was found to be more effective. The bishkatali (*Polygonum hydropiper* L.), garlic (*A. sativum* L.), ginger (*Zingiber officinale* Rose) and neem (*Azadirachta indica* A. Juss) extracts were effective against *A. alternata* (Rahman *et al.*, 1999) ^[7]. Ranjan *et al.* (1999) ^[8] reported that out of five plant extracts tested against *Alternaria alternata in vitro*, *Aegle marmelos* leaf extract (5000 µg/ml) showed minimum colony diameter and maximum growth inhibition of *A. alternata* in comparison

with the control. All the plant extracts were less effective at lower concentrations, there was a positive correlation between concentration and growth inhibition percentage. The bulb extracts of *A. sativum* and leaf extracts of

Ocimum silicum L. at two per cent were most effective in suppressing radial growth and biomass production of *A. alternata* (Rashmi and Yadav, 1999) [9].

Table 3: Effect of cow products and botanicals on Colony diameter, colour, type of colony and Sporulation

Symbol	Treatments	Concentrations						Mean (A)	Colour	Type of colony
		10%		20%		25%				
		Diameter (mm)*	Sporulation	Diameter (mm)*	Sporulation	Diameter (mm)*	Sporulation			
T ₁	<i>Azadiracta indica</i> (leaf) extract	52.98	++	43.46	+	32.69	+	43.04	Greyish black	circular
T ₂	Garlic bulb extract	54.23	++	44.96	+	35.46	+	44.88	Greyish black	circular
T ₃	Cow dung	64.83	+++	52.58	+++	48.33	++	55.25	Greyish black	circular
T ₄	Cow urine	55.81	++	45.76	++	38.43	+	46.67	Greyish	Irregular
T ₅	Milk whey	62.46	+++	50.87	++	46.28	++	53.20	Greyish	Irregular
T ₆	<i>Calotropis gigantean</i> (leaf) extract	58.64	++	48.26	++	42.15	+	49.67	Greyish black	circular
T ₇	Control	74.52	+++	74.52	+++	74.52	+++	74.52	Greyish black	circular
Mean (B)		60.49		51.48		45.40		52.45	—	—
Factor			SEm(±)				C.D. at 5%			
Factore (A)			0.23				0.66			
Factore (B)			0.15				0.43			
Factore (A X B)			0.40				1.149			

Table 4: Growth Inhibition of mycelium under *in-vitro* condition

S. No.	Symbols	Treatment	Colony growth (mm)	Inhibition (%)
1	T ₁	<i>Azadiracta indica</i> (leaf) extract	43.04	42.24
2	T ₂	Garlic bulb extract	44.88	39.77
3	T ₃	Cow dung	55.25	25.86
4	T ₄	Cow urine	46.67	37.37
5	T ₅	Milk whey	53.20	28.61
6	T ₆	<i>Calotropis gigantean</i> (leaf) extract	49.67	33.35
7	T ₇	Control	74.52	00.00
SEm (±)			5.99	
C.D. at 5%			18.16	

Disease severity (%) and Disease reduction (%)

Table -5 showed that severity of *Alternaria* leaf spot in cow products and botanicals were lower than control plot. The ranges of Disease severity (%) of plots were 43.33 to 66.66% founded in trial field. The revelatory minimum Disease severity (%) was found in *Azadiracta indica* (leaf) extract is 46.11%, followed by Garlic bulb extract is 48.33% and Cow urine 49.99% while maximum Disease severity (%) was found in cow dung is 56.66% followed by Milk whey is 53.33% and *Calotropis gigantean* (leaf) extract 52.22%.

The effect of cow products and botanicals are reducing the disease severity and increase disease reduction (%) over control plot. Maximum disease reduction (%) was recorded in *Azadiracta indica* (leaf) extract is 32.52% followed by Garlic bulb extract is 29.27% and Cow urine is 26.84% while minimum disease reduction (%) was recorded in cow dung is 17.08% followed by Milk whey is 21.95% and *Calotropis gigantean* (leaf) extract is 23.58%. In accordance to the present findings of Jandaik *et al.* (2015) [3] who confirmed and determined antifungal activity of cow urine at various concentrations viz., 5, 10 and 15% against pathogen that were purified from infected plants of both

Fenugreek, Okra. Among all, cow urine @ 15% was found most effective. When their inhibitory effects were evaluated, highest inhibition was examined against *F. oxysporum* (78.57%) @15% of cow urine followed by *R. solani* (78.37%) and *S. rolfsii* (73.84%). Present findings are also related to findings of Patni *et al.*, (2005) [6] who reported that six medicinal plants were evaluated for inhibitory effect against *A. brassicae* causing blight in mustard. Among all, *Calotropis*, *Eucalyptus* and *Polyalthia* extracts, had shown significant effect in reducing the growth of the pathogen.

Table 5: Effect of cow products and botanicals on *Alternaria* leaf spot severity and disease reduction under *in-vivo* conditions

Symbol	Treatment	Disease severity (%)	Disease reduction (%)
T ₁	<i>Azadiracta indica</i> (leaf) extract	46.11	32.52
T ₂	Garlic bulb extract	48.33	29.27
T ₃	Cow dung	56.66	17.08
T ₄	Cow urine	49.99	26.84
T ₅	Milk whey	53.33	21.95
T ₆	<i>Calotropis gigantean</i> (leaf) extract	52.22	23.58
T ₇	Control	68.33	0
SEm(±)		2.3	2.5
C.D. at 5%		7.1	7.7

Conclusion

In the *In vitro* condition, *Alternaria alternata* was observed Greyish black colony colour and circular type colony growth while three cow products and three botanicals were tested and *Azadiracta indica* (leaf) extract and Garlic bulb extract found more effective than other treatments. In the *In vivo* condition, least Disease severity and maximum disease reduction was found in *Azadiracta indica* (leaf) extract and minimum reduction in cow dung than other cow products and botanicals was recorded.\

References

1. Anonymous. All India Coordinated Project on Cotton, Project Coordinator Report. 2019. Available from: http://www.aicrip.cicr.org.in/CD_18_19/2-PC-Report.pdf
2. Hassanein NM, Abou Zeid MA, Youssef KA, Mahmoud DA. Control of tomato early blight and wilt using aqueous extract of neem leaves. *Phytopathologia Mediterranea*. 2010;49(1):143-151.
3. Jandaik S, Thakur P, Kumar V. Efficacy of cow urine as plant growth enhancer and antifungal agent. *Advances in Agriculture*. 2015.
4. Karade VM, Sawant DM. Screening of various plant extracts against *Alternaria alternata* (Fr.) Keissler. *Journal of Maharashtra Agricultural University*. 1999;24:311-312.
5. Panchal DG, Patil RK. Eco-friendly management of fruit rot of tomato caused by *Alternaria alternata*. *Journal of Mycology and Plant Pathology*. 2009;39(1):66-69.
6. Patni CS, Kolte SJ, Awasthi RP. Efficacy of botanicals against *Alternaria blight* (*Alternaria brassicae*) of mustard. *Indian Phytopathology*. 2005;58(4):426-430.
7. Rahman GMM, Islam MR, Wadud MA. Seed treatment with plant extracts and hot water, a potential biophysical method of controlling seed borne infection of wheat. *Bangladesh Journal of Training and Development*. 1999;12:185-190.
8. Ranjan S, Lal HC, Ojha KL. Efficacy of plant extracts against *Alternaria* leaf blight of sunflower *in vitro*. *Journal of Applied Biology*. 1999;9:93-94.
9. Rashmi, Yadav BP. A comparative efficacy of fungicides and plant extracts on radial growth and biomass production of *Alternaria alternata*. *Journal of Applied Biology*. 1999;9:73-76.
10. Sheo Raj. Grading for Cotton Diseases. Bulletin, Central Institute for Cotton Research, Nagpur, India. 1988. p. 1-7.
11. Srinivasan KV. Cotton diseases. Indian Society for Cotton Improvement, Central Institute for Cotton Research, Mumbai. 1994. p. 157-311.
12. Turkusay H, Onogur F. Studies on antifungal effects of some plant extracts *in vitro*. *Turkish Journal of Agriculture and Forestry*. 1998;22:267-271.
13. Watkins GM. Compendium of Cotton Diseases. American Phytopathological Society Publishers.
14. Zaib P, Iqbal M, Shahzadi R, Ahmad HM, Rasool B, Khan SA, Ansari MR. Introductory chapter: Recent trends in cotton research. 2020. doi:10.5772/intechopen.88776.