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## Morphological and cultural studies of *Alternaria alternata* causing leaf spot and fruit rot of chilli

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### Abstract

Leaf spot and fruit rot of chilli caused by *Alternaria alternata* (Fr.) Keissler is one of the most destructive disease of chilli (*Capsicum annum* L.). Study was undertaken to conduct collection of samples, isolation, identification, pathogenicity, morphological and cultural characters of pathogen on different culture media *in vitro*. The isolations were made from the symptomatic samples collected from chilli plot from All India Coordinated Research Project on Vegetable Crops, Mahatma Phule Krishi Vidyapeeth, Rahuri. The isolation of fungal pathogen was done from affected samples of chilli leaf. Further purified and maintained on PDA media. Pathogenicity of test fungal pathogen was proved on healthy chilli plant in earthen pots under glasshouse conditions. The pathogen was identified as *Alternaria alternata* on the basis of morphological and cultural characteristics. The pathogen showed typical symptoms of leaf spot on chili in glasshouse. *Alternaria alternata* showed spots with concentric rings on leaves. *Alternaria* causes small reddish purple spots most characteristic symptom was brown to dark spots on older leaves. The circular spots enlarge and soon develop dark, concentric rings or ridges gaved target-like appearance. Lesion growth stopped by major leaf veins. Spotted leaves soon turn yellow, wither and died. In cultural studies, it was observed that the fungal test pathogen *Alternaria alternata* showed maximum radial growth and excellent sporulation on PDA media.

**Keywords:** *Alternaria alternata* (fr.) keissler, culture media, *in vitro*, leaf spot and fruit rot, concentric rings, sporulation, fungal colony, muriform

### Introduction

India, being the largest chilli producer, has vast potentiality to increase production and promote export besides meeting its domestic requirements. However, despite continuous efforts at various levels, the chilli productivity did not gain accepted momentum. This could be attributed to various biotic and abiotic constraints. Among the biotic stress fungus causing most common widely distributed diseases. Chilli attacked by a large number of fungal diseases depending upon the locality and agro climatic conditions.

Leaf spot and fruit rot disease caused by *Alternaria alternata* (Fr.) Keissler is becoming a limiting factor and posing a major problem in chilli production. The pathogen has been reported to cause seed, seedling, leaf and fruit diseases as well as Post harvest decay of fruit and seed has also been reported due to this pathogen. *Alternaria* leaf spot fungus, survives in or on seed as tiny black stromata in old affected leaves in the soil. Infection occurs by direct penetration to the leaf. *Alternaria* spores required high humidity in the atmosphere for germination and penetration to the host; however, heavy dew is sufficient for infection of the pathogen. The disease is most severe during periods of warm temperatures and excessive moisture either from rain or overhead irrigation. The fungus is spread by splashing water, wind, and leaf-to-leaf contact.

### Material and Methods

#### Collection of diseased sample and isolation of pathogen

The disease sample was collected from the infected chilli plot Research Project on Vegetable Crops, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra, India. Samples showing typical symptoms were used for isolation of pathogens under study and the culture obtained was used for further investigation.

The common laboratory medium i.e., Potato dextrose agar (PDA) media was used for isolating the fungal pathogen responsible for leaf spot and fruit rot of chilli. The organism repeatedly sub-cultured on potato dextrose slants for maintaining the pure culture for further studies.

### Morphological Characters

Morphological characters such as mycelia width, length and width of conidia, colony colour, and type of margin, colour of margin, mycelial growth and sporulation were measured under compound microscope. The length, breadth, colour and number of septations and the shapes of spore were also recorded.

### Cultural Characters

The variation in cultural characters of *Alternaria alternata* was studied on the different solid media and the best media for the fungus growth was identified. The growth characters of the fungus were studied on eight different solid media. All the media were sterilized at 121 °C, at 15 pounds pressure for 15 minutes. Twenty ml of each of the medium was poured in to 90 mm diameter petridishes. Such plates were inoculated with five mm disc of fungal growth and incubated at 27 ± 1°C. Each treatment was replicated thrice. Colony diameter was recorded by averaging the linear growth of the colony in two directions for each plate after 5, 7, and 9 day of inoculation. The fungal colony colour, surface elevation and sporulation were also recorded at the end of the incubation period. The data on radial growth was analysed statistically. The different media used in the studies viz., PDA media, Oat meal agar, Corn meal agar, Richard's medium, Martin's medium, V-8 juice agar, Host leaf dextrose agar, and Carrot extract agar medium.

## Result and Discussion

### Morphological Characters

#### 1. Mycelium

The fungus produced profuse mycelial growth on PDA. Initially, the mycelium was hyaline that turned to grey-brownish, multi celled, septate and irregularly branched. In early growing stage, hyphae were thin, narrow and hyaline but became slightly thick as they grew old.

#### 2. Conidia

Conidia were born in chains up to 10 or more on conidiophores. They were light olivaceous to dark brown in colour, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal and 2-10 transverse septa. The muriform conidia inclusive of beak measured 47.16 µm (21.82 - 96.40 mm) x 8.26 - 16.52 µm (13.49 mm). The length of the conidium was 3-5 times (2.64 to 5.84 times) more than its width. The beak measured 27.12 µm (22.62 - 58.69 µm) in length.

#### 3. Conidiophores

Conidiophores arised singly or in clusters, usually 2-6 and were long or short. They were pale olivaceous to olivaceous brown, straight or curved, geniculate, slightly swollen at apex having terminal scars indicating the point of attachment of conidia. The conidiophores measured 41.12 µm (26.20-114 µm) in length and 4.31 µm (3.25-8.21 µm) in width. The

length: width ratio was 9.85 (8.75 -13.29).

### 4. Chlamydo spores

The chlamydo spores were formed in the old culture of *A. alternata*. They were intercalary, thick walled, roundish to oval in shape, dark brown in colour and measuring 7.22 µm (4.92 - 9.89 µm) in diameter.

Similar result was obtained by Nagrale *et al.*, (2013) [6] who recorded morphological observations of *A. alternata* by adopting slide culture technique. Devappa and Thejakumar (2016) [2] studied that the culture of the fungal colony was initially white, cottony with profuse aerial mycelium which gradually turned grey colour. Aged culture appeared completely greyish with aerial mycelium and distinct concentric rings was formed on medium. Conidiophores were short to long, simple or branched arising singly. Conidiophores were golden to brown coloured with 2-9 transverse and 0-2 longitudinal septa. Conidia were born in long chains (6-11) on conidiophores, they were thick walled, beaked and brown in colour.

### Cultural characters

The variation in cultural characters of *Alternaria alternata* was studied on the eight different solid media. And the results thus obtained are presented in Table 1, 2 and Fig. 2. The radial growth, colony characters and sporulation of the fungi were recorded, when the maximum growth was attained on any one of the tested media. The effect of different culture media on the growth of fungi differed significantly. Maximum radial growth of *A. alternata* was recorded on PDA media (88 mm), which was found to be significantly superior to all other media followed by Richard's medium (85 mm), V-8 juice agar (77.50 mm), Martin's and Carrot extract agar (75 mm), Corn meal agar (70 mm) and Oat meal agar (65 mm). The least radial growth was recorded in Host leaf dextrose agar media (37.50 mm). Mycelium colour varied from grayish to brownish. The growth varied from flat to raised and fluffy to sparse. Pigmentation in the media also varied from brown, pinkish, white and black. Sporulation also showed greater variation in different media, ranging from excellent to poor. Excellent sporulation was recorded on PDA Media, good sporulation in Oat meal agar, Carrot extract agar, and Richard's medium, moderate sporulation in Corn meal agar and Host leaf dextrose agar, poor sporulation was recorded in Martin's medium while sporulation was not recorded in V-8 media.

**Table 1:** Growth of *Alternaria alternata* on different solid media

Sr. No.	Media	Mean colony diameter (mm)*		
		5 DAI	7 DAI	9 DAI
1.	PDA Media	50	65	88
2.	Oat meal agar	53	60	65
3.	V-8 juice agar	41	61	77.50
4.	Carrot extract agar	50	60	75
5.	Corn meal agar	55	65	70
6.	Richard's medium	60	75	85
7.	Martin's medium	61	67	75
8.	Host leaf dextrose agar	20	25	37.50
	S.E. ±	0.86	0.90	0.95
	CD at 5%	2.58	2.70	2.87
	CD at 1%	3.55	3.75	3.95

\* Each value is the mean of three replications, Note: DAI: Days after inoculation

**Table 2:** Cultural characteristics and sporulation of *Alternaria alternata* on different solid media after 9 days of incubation

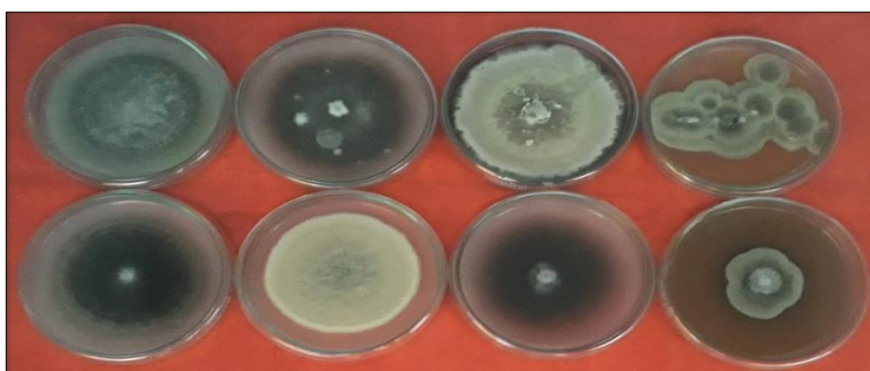
Sr. No.	Media	Growth characteristics	Sporulation
1.	PDA Media	Brownish, fluffy over growth with regular margins and concentric rings.	++++
2.	Oat meal agar	Blackish growth with irregular margins, good mycelia growth	+++
3.	V-8 juice agar	Brownish in colour, irregular growth.	-
4.	Carrot extract agar	Dark brownish in colour, uniform, concentric rings with uniform margin.	+++
5.	Corn meal agar	Dark brown to black, uniform and regular margins.	++
6.	Richard's medium	Greyish, fluffy growth black with margin.	+++
7.	Martin's medium	Dull greyish, fluffy growth with regular and circular margin.	+
8.	Host leaf dextrose agar	Greyish, fluffy over growth with irregular margin, with slow mycelia growth.	++

Here, - = No sporulation, + = Poor sporulation, ++ = Moderate sporulation, +++ = Good sporulation, ++++ = Excellent sporulation



(A) Pure culture (B) Mycelium and spores (C) Conidiophore

**Fig 1:** Pure culture (A) and microphotograph (B, C) of *Alternaria alternata*



1. PDA media, 2. Corn meal agar, 3. Richard's medium, 4. V-8 juice agar, 5. Carrot extract agar, 6. Martin's medium, 7. Oat meal agar, 8. Host leaf dextrose agar

**Fig 2:** Growth of *Alternaria alternata* on different solid media

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