

Original Research Article

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Morphological, Cultural and Pathological Variation of *Alternaria alternata* causing Leaf Spot of Niger

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ABSTRACT

Keywords

Alternaria alternata, Niger, *Guizotia abyssinica*, Leaf spot, Cultural and Morphological characterization

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Leaf spot disease (*Alternaria alternata*) causes severe foliar damage to niger crop. The isolates of *A.alternata* were collected from different location and tested for their variability in term of morphological, cultural and pathological variation viz., growth rate, colony colour, aerial mycelium colour, growth pattern, sporulation, conidial size, conidial separation. In case of growth rate Aa1 and Aa2 was observed very fast growing it cover 70-73 mm growth within 7 days of inoculation on PDA media. Among the five isolates different colony colour and aerial mycelium colour was observed. The variation in mycelial growth pattern was observed in Aa2 isolate growing with circular pattern and other four show irregular growth pattern. In respect of sporulation abundant sporulation was observed in Aa2 show more than 30 spore in single microscopic field. In case of conidial size maximum conidial length (41.10) was noticed in Aa5 and breadth (11.66) in Aa1. The Maximum number of transverse septa were observed in Aa5 (4-8) and longitudinal septa in Aa1 and Aa2 (0-3). In case of pathogenicity Aa2 isolates was most virulent than other isolates of *Alteranria alternata*.

Introduction

Niger [*Guizotia abyssinica*, family: compositae] is commonly known as ramtil. It is an annual dicotyledonous herb Plant erect, stout and branched type, flowers are yellow, slightly green and two to three capitulates grow together. It is cross pollinated and minor, traditional oil seed crop in India. It is important minor, edible, traditional oil seed crop in India. It is mainly cultivated in tribal pockets of Gujarat, M.P, Orissa, Maharashtra, Bihar, Karnataka and Andhra Pradesh in India

(Gupta, 2018). India ranks first in area, production and export of niger in the world (Rai *et al.*, 2016). It provides about 3 per cent of the edible oil requirement of the country (Getinet and Sharma, 1996). Niger contains 34-36% quality oil with 18-20 % protein in the seed (Rai *et al.*, 2016).

Niger oil has good keeping quality and has < 70% unsaturated fatty acids free from toxin. linoleic acid 45-66%, oleic acid 13-39 % and palmitic acid 8.2-9.6%.It has good potential for soil conservation, land rehabilitation and

as a biofertilizer. These attributes favour its cultivation on hilly areas, marginal and submarginal lands in and around the forest (Ranganatha *et al.*, 2009). Nigeris suffering from various fungal disease among them *Alternaria* leaf spot is distributed throughout the Niger growing areas of India. Leaf blight of niger caused by *Alternaria alternata* is considered to be a major devastating disease to the Niger in India and also reduce the yield and oil quality (Nagaraja and Krishnappa 2016).

The disease is favoured by warm and humid climate. The accidental rain at flowering stage leads the expansion of *alternaria* leaf spot incidence and results in the poor seed set and seed yield (Sandipan *et al.*, 2014). Diseases cause heavy damage upto 35-40 per cent to this crop and reduce its seed yields upto 20-30 per cent.

Materials and Methods

Isolation and Identification of *A. alternata*

The present investigation on morphological and cultural variability among the isolates of *Alternaria alternata* was conducted at the Department of Plant Pathology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth (Dr. PDKV), Akola. In order to isolate pathogen infected leaf sample were cut along with healthy leaf and surface sterilized with 0.1% sodium hypochlorite solution for one minute and washing with three times by sterilized distilled water. The bits were placed in petriplates containing PDA medium. The plates were incubated at $27 \pm 2^{\circ}C$ for 7 days. All the above operations were carried out in sterilized condition (under laminar air flow unit). The isolated fungi were identified as *Alternaria alternata* on the basis of morphological characters and published literature. The fungal culture was purified by following hyphal tip method and culture

obtained was maintained on potato dextrose agar (PDA) medium slants by adopting subsequent sub culturing at regular intervals.

Morphological and cultural characteristics

The growth characters of different isolates of *Alternaria alternata* were studied on PDA media morphological characters *viz.*, mycelium growth rate cultural characters *viz.*, colony colour, aerial mycelium colour, growth pattern, sporulation, size of conidia, and septation of conidia.

Pathogenicity test

Three weeks old healthy niger seedling were selected for inoculation selected seedlings surface disinfect with sodium hypochloride for two minutes and then with water. Conidial suspension was prepared which having (1×10^6 spore/ml). The selected seedling first injured with carborandom powder then were inoculated with conidial suspension with the help of hand sprayer.

After inoculation the inoculated crop were placed in green house, where relative humidity 80-90% and optimum temperature ($27 \pm 1^{\circ}C$) maintained for further development of symptoms of *alternaria* leaf spot. Initiation of typical symptoms after 6-8 days on the healthy leaf was observed. The diseased portion cut into small pieces along with healthy part and the pathogen was reisolated on the PDA medium from the inoculated plants for confirmation of Koch's postulates.

Results and Discussion

Growth rate variability among the isolates *Alternaria alternata*

The isolates Aa2 and Aa1 was observed to be very fast growing it cover 70-74 mm mycelium growth within seven days of

incubation. While isolates Aa5 and Aa4 are considered fast growing with mycelial growth 65-70mm 7 days of incubation.

The Aa3 isolates from Deulgaon Mahi (Buldhana) was slow growing with 61.5 mm growth within 7days. These results are in confirmity with the finding of Singh *et al.*, (2014) who observed variation in radial

mycelial growth of *Alternaria helianthi* on PDA. Ginoya and Gohel (2015) and Jankar *et al.*, (2017) also recorded variation in mycelial growth of *Alternaria alternata* on different media. In the present investigation also the varied mycelial growth rate was recorded during three to seven days of incubation of *A. alternata* isolates obtained from leaf spot disease of niger.

Table.1 Location wise naming of isolates and growth rate of various isolates of *Alternaria altenata* on PDA

Sr. No.	Location	Isolates	Radial mycelial growth (mm)*			Mycelial growth rate
			3 rd DAI	5 th DAI	7 th DAI	
1.	Sindakhed Raja	Aa1	26.50	51.00	70.00	Very fast
2.	Chikhali	Aa2	29.50	54.00	73.00	Very fast
3.	Deulgaon Mahi	Aa3	20.50	40.00	61.50	Slow
4.	Department of Plant Pathology Dr. PDKV, Akola	Aa4	27.50	52.00	66.50	Fast
5.	Department of Agronomy Dr. PDKV, Akola	Aa5	22.50	50.00	66.00	Fast

DAI- Days after Inoculation

Table.2 Cultural characters of the test isolates of *Alternaria alternata*

Isolates	Colony colour	Aerial mycelium colour	Growth pattern	Sporulation category
Aa1	Greyish black	Dull white	Irregular	3
Aa2	Blackish colour	Dark grey	Circular,Concentric	4
Aa3	Greenish brown	Light brown	Irregular	2
Aa4	Dark brown	Light brown	Irregular	3
Aa5	Blackish green	Dull white	Irregular	3

Categories of sporulation given by Ginoya and Gohel (2015) for *Altenaria alternata*

Rate of sporulation	No. of spore /microscopic field (45x)	Sporulation category
Abundant	>30	4
Good	20-30	3
Moderate	10-20	2
Scanty	<10	1
Nil	0	0

Table.3 Size and separation of conidia of *Alternaria alternata* on PDA

Isolates	Size of conidia (µm)		Separation of conidia	
	Length	Breadth	Transverse	Longitudinal
Aa1	21.75	11.62	1-4	0-3
Aa2	23.75	11.52	2-6	0-3
Aa3	37.75	3-7	0-2	
Aa4	20.30	11.30	2-4	0-2
Aa5	41.10	10.20	4-8	0-1

Table.4 Pathological variation in the isolates of *Alternaria alternata*

Sr.No.	Isolates	Days to initiate disease symptoms	Average No. of lesions / plant
1.	Aa1	8	6
2.	Aa2	6	8
3.	Aa3	10	4
4.	Aa4	9	4
5.	Aa5	12	3

Fig.1 Growth of *Alternaria alternata* isolates on PDA and conidia

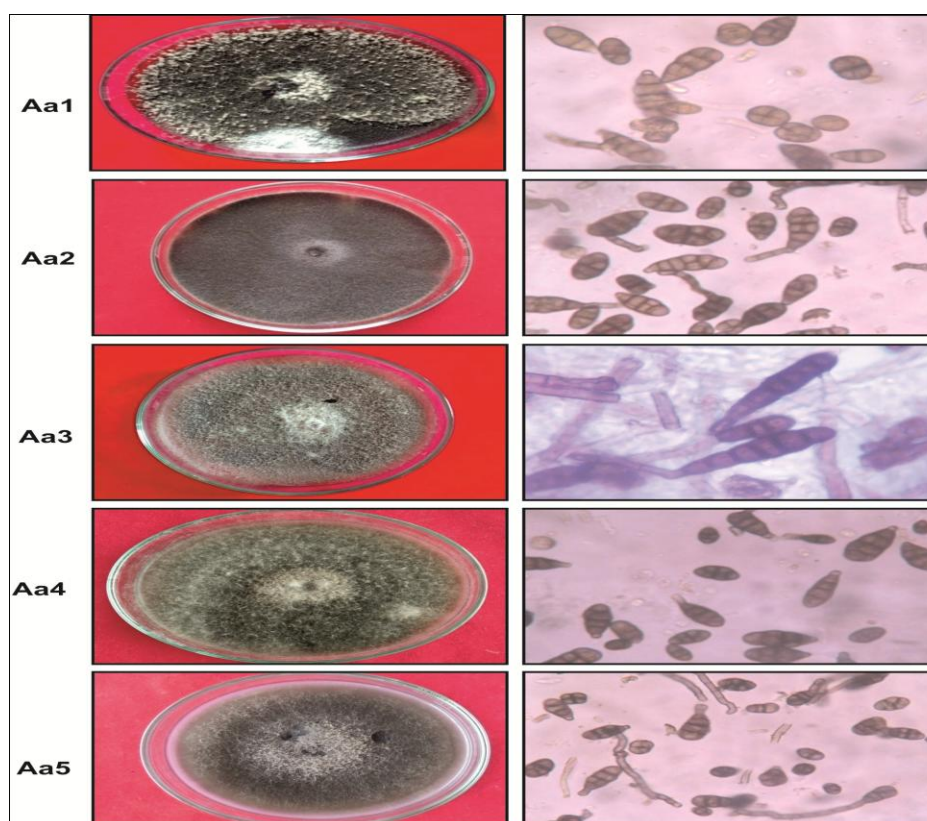
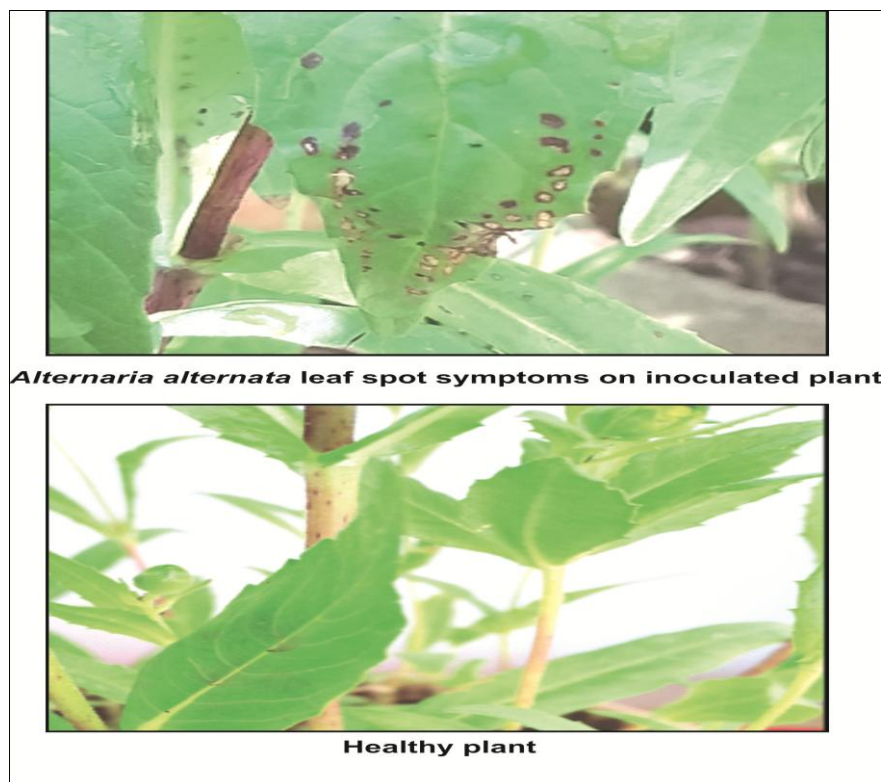


Fig.2 Pathogenicity test of *Alternaria alternata*



Cultural characters of the test isolates of *Alternaria alternata*

The result is given in table 2. revealed that there was a considerable variation among colony colour on PDA medium. Among the different isolates greyish black colour was observed in Aa1 isolate. However Black colour in Aa2, greenish brown colour in Aa3, dark brown in Aa4 and greenish black colour in Aa5 isolates after seven days of incubation on PDA. The isolates Aa1 and Aa5 showed dull white colour of aerial mycelium on PDA. Similarly Aa3, Aa4 showed light brown and Aa2 showed dark grey colour. The variation in mycelial growth patterns among various isolates were also observed Aa2 growing with circular pattern of margin and Aa1, Aa3, Aa4 and Aa5 isolates were growing with irregular pattern of margin. The abundant sporulation was observed in Aa2 having more than 30 spore in single microscopic field followed by

Aa1, Aa4, and Aa5 isolates which showed good sporulation having 20-30 spore in single microscopic field. Isolates Aa3 showed moderate sporulation. The present result are in agreement finding Jankar *et al.*, (2017) who recorded variation in colony colour and sporulation among the isolates of *Alternaria alternata*. Result on the same line was also recorded by Rajendra *et al.*, (2013) and Devappa and Thejakumar (2016) recorded variation in colour of aerial mycelium, growth pattern and sporulation.

Size and septation of conidia of *Alternaria alternata* on PDA

The result presented in table 3. Indicates distinct variation in size and septation of conidia. Among the five isolates, the maximum conidial length (41.10 μ m) was noticed in Aa5, followed by (37.75 μ m) in Aa3 while, the minimum conidial length

(20.30 μ m) was noticed in Aa4 isolate. The maximum conidial breadth (11.62 μ m) was recorded in Aa1 isolate followed by (11.52 μ m) in Aa2. While the minimum conidial breadth (10.20 μ m) was noticed in Aa5 isolate. Conidial septation as presented in table 3. there was difference among the isolates with respect to transverse and longitudinal conidial septation. The maximum number of transverse septa was observed in Aa5 (4-8) isolate and minimum (1-4) in Aa1 isolate. The maximum number of longitudinal septa was observed in Aa1 and Aa2 (0-3) and minimum in Aa5 (0-1) isolate. Overall average number of septation among the isolates varied from 1-8 transverse and 0-3 longitudinal septa. These conidial features of *Alternaria alternata* observed in the study are in accordance with findings of Monowara *et al.*, (2017) who reported conidial morphological differences among the isolates of *Alternaria spp.* in terms of length, breadth and number of septa. Ginoya and Gohel (2015) and Jankar *et al.*, (2017) also reported similar variation in the conidial morphology of the isolates of *Alternaria alternate* (Fig. 1).

Pathogenicity of *Alternaria alternata* on host plant

The initiation of typical symptoms appears after 5-10 days of inoculation. Formation of small necrotic lesions concentric rings on leaves, which turn brown to black in colour in advance stage they become circular to irregular in shape, was observed in (Fig. 2). The pathogen was successfully reisolated from artificially inoculated diseased plant. On comparison, reisolated culture were of identical with the pure cultures of the pathogen initially isolated from naturally infected plant which confirmed the Koch's postulates and pathogenic ability of isolated pathogen *Alternaria alternata*. Various earlier worker have been reported the pathogenic nature of *Alternaria alternata* in different

crops. Kansara and sablpar (2015) proved the pathogenicity of *Alternaria alternata* causing leaf spot of niger. Jankar *et al.*, (2017) and Devappa and Thejakumar (2016) were also ascertained the pathogenicity of *Alternaria alternata* in chilli by proving Koch's postulates.

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