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Spatial distribution and characterization of *Alternaria alternata* causing leaf spot in blackgram

Ambarish KV[1*], Adinarayana M[2], Anil kumar P[1], Madhumathi T[3]
[1] Department of Plant Pathology, College of Agriculture, Bapatla, Andhra Pradesh, India
[2] Regional Agricultural Research Station, Lam, Guntur, Andhra Pradesh, India
[3] Department of Entomology, College of Agriculture, Bapatla, Andhra Pradesh, India

Abstract
Blackgram is a short duration legume crop affected by fungi, bacteria, viruses and nematode. Among the fungal diseases, Alternaria leaf spot disease caused by the *Alternaria alternata* (Fr.) Keissl. is becoming a major problem in blackgram fields of Andhra Pradesh. The survey conducted during *rabi* 2017-18 revealed that Alternaria leaf spot disease severity was more or less same in both the mandals surveyed. The disease kept on increasing with increase in the age of the crop. The disease progressed from 6.49% at 40 DAS to 17.89% at 50 DAS and further to 50.62% at 60 DAS in Ponnur mandal. In Amruthuluru mandal, the disease progress was 5.49% at 40 DAS to 19.16% at 50 DAS and 51.29% at 60 DAS. The pathogen isolated from the leaf spot symptom was identified as *A. alternata* by comparing with standard descriptions made by previous reports based on characteristics of the colony, hyphae, conidiophore and conidia.

Keywords: blackgram, *alternaria alternata*, survey, disease severity, characterization

Introduction

Blackgram or *urdbean* [*Vigna mungo* (L.) Hepper], is one of the important pulse crops which is grown as a source of nutrition and income to billions of people in South East Asia. The production of blackgram is mostly confined to Asian countries, of which India is the largest producer followed by Myanmar and Thailand. In India it occupies in an area of 36.24 lakh ha producing 19.45 lakh tons with a productivity of 537 kg/ha (Anon., 2018) [1]. Major blackgram growing states in India are Andhra Pradesh (A.P.), Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Sikkim, Tamil Nadu and Uttar Pradesh. It is mainly cultivated under rice fallows in coastal A.P. in an area of 4.56 lakh ha, with a production and productivity of 4.11 lakh tons and 901 kg/ha, respectively (Anon., 2018) [1].

Consistent yields were not reported in blackgram and in some seasons a marked decline was reported due to its susceptibility to several fungal and viral diseases as well as pests. Out of different constraints, fungal diseases play major role in affecting the crop and yields. Important among the diseases are powdery mildew caused by *Erysiphe polygoni* and rust caused by *Uromyces appediculatus*, leaf spots caused by *Corynespora cassicola and Cercospora canascens.* Of late, *Alternaria* species causing leaf spot and blight in blackgram is becoming endemic and appearing in severe form. So for not many studies were conducted in blackgram on this disease regarding its prevalence, distribution and characterization. Hence, the present study is planned for this objective.

Materials and methods

Survey

Survey was undertaken during *rabi* 2017-18 season in major blackgram growing mandals of Guntur district, Andhra Pradesh. Based on crop statistics of preceeding year, two fields from each village, two villages from each mandal and two mandals from the district were chosen and survey was conducted. In each field five square meter area (one square meter each) were selected. Four from each of the four corners, leaving the border rows and another at the centre to record the severity of Alternaria leaf spot disease. Disease severity was assessed by following disease rating scale given by Alice and Nadarajan, 2007 [3] (Table 1).

Table 1: Disease scale for Alternaria leaf spot (1-9 scale)

Grade	Description	Reaction type
1	Free from diseases	Immune
2	Traces to pin head size spots on leaves	Highly resistant
3	Spots slightly larger than pin heads	Resistant
4	Spots occupying 2-5 % leaf area	Moderately resistant
5	Spots occupying 5-10 % leaf area	Moderately susceptible
6	Spots occupying 10-25 % leaf area	Susceptible
7	Spots occupying 25-50 % leaf area	Susceptible
8	Spots occupying 50-75 % leaf area	Highly susceptible
9	Spots occupying more than 75 % leaf area	Highly susceptible

Per cent disease index (PDI) for Alternaria leaf spot disease was calculated by using the following formula (Wheeler, 1969) [13].

$$PDI = \frac{\text{Sum of individual disease ratings}}{\text{No. of observations assessed X maximum disease rating}} X100$$

Isolation and characterization of the Causal Organism

Isolation of the Causal Organism

A. alternata was isolated from the infected blackgram leaves of LBG 752 collected from the field during survey. Small bits of

0.5 to 1.0 cm size were cut from the diseased area of the infected leaf along with some healthy portion, surface sterilized with 1.0 % sodium hypochlorite for 30 seconds and rinsed in three changes of sterilized distilled water. The surface sterilized bits were transferred on to PDA medium contained in sterilized Petri dishes aseptically and incubated at room temperature (25 ± 1^0C) in the laboratory. Five days after incubation, when the growth of the fungus was seen on the medium, it was transferred aseptically to PDA slants.

Characterization of the pathogen

The culture of *A. alternata* was identified based on the characteristics of the colony, hyphae, conidiophore and conidia. Length, breadth and beak length were measured for 25 conidia and compared with the dimensions of *A. alternata* reported by earlier workers.

The conidial measurements were made with Labomed LX 400 microscope with progress capture pro 2.5 version software (Labo America Inc. Ferment, California, USA). The identity of the fungus was established by associating the ranges of dimensions made by the following formula (John, 1970) [7] with the regular dimensions for the pathogens available in literature.

$\mu = \chi \pm t\ 0.05$ (SE)

$SE = \sigma / \sqrt{\eta}$

Where

σ = Standard deviation

μ = Population mean

χ = Sample mean

η = Number of spores observed

t = Table t value (P=0.05)

SE = Standard error.

Maintenance of the Culture

A. alternata culture was sub-cultured on PDA slants and allowed to grow at 25 ± 1^0C for ten days and the slants with fungal culture were preserved in a refrigerator at 5^0C and when needed, the culture was used after restoring it to its active stage by keeping it at room temperature.

Results and discussion

Occurrence

Survey for Alternaria leaf spot severity was taken up in farmer's field. Survey was done in two villages in each of the two selected mandals, *i.e.*, in Ponnur and Amarthalur mandals of Guntur district during *Rabi* 2017-18. Disease severity was assessed at five locations in each field surveyed. Alternaria leaf spot disease severity was recorded at 10 days interval starting from 30-35 DAS to 60-70 DAS (Table 2).

The overall mean of Alternaria leaf spot severity in Guntur district was 0% at 30-35 DAS, 5.99% at 40-45 DAS, 18.53% at 50-55 DAS and 50.96% at 60-70 DAS (Table 2).

Lack of disease occurrence up to 30-35 DAS may be attributed to higher sugar content in leaves. Horsfall and Dimond (1957) [6] and Bhargava and Khare (1988) [4] reported that Alternaria is a low sugar pathogen and occurs at or after flowering.

In the present investigation disease occurrence noticed at 40-45 DAS, *i.e.*, at flower initiation when sink was established.

Alternaria disease severity was more or less same in both the mandals surveyed. The disease kept on increasing with increase in the age of the crop.

The disease progressed from 6.49% at 40 DAS to 17.89% at 50 DAS and further to 50.62% at 60 DAS in Ponnur mandal. In Amarthalur Mandal, the disease progress was 5.49% at 40 DAS to 19.16% at 50 DAS and 51.29% at 60 DAS (Table 2).

At 40-45 DAS, the Alternaria leaf spot severity in the surveyed villages ranged from 5.16% (Sangupalem kooluru village of Ponnur mandal) to 7.83% (Chintalapudi village of Ponnur mandal).

At 50-55 DAS, the Alternaria leaf spot severity was in the range of 16.81% (Sangupalem kooluru village of Ponnur mandal) to 20.45% (Govada village of Amarthalur mandal). At 60-70 DAS the Alternaria leaf spot severity was in the range of 49.38% (Inturu village of Amarthalur mandal) to 53.20% (Govada village of Amarthalur mandal) (Table 2).

Table 2: Severity of blackgram Alternaria leaf spot disease in Guntur district of A.P. during *rabi*, 2017-18

Mandal	Village	Severity Of Alternaria Leaf Spot (PDI)			
		30-35 DAS*	40-45 DAS	50-55 DAS	60-70 DAS
Amarthalur	Govada	0	5.34	20.45	53.2
	Inturu	0	5.65	17.87	49.38
	Mandal mean	0	5.49	19.16	51.29
Ponnur	Sangupalem Kooluru	0	5.16	16.81	50.14
	Chintalapudi	0	7.83	18.98	51.12
	Mandal mean	0	6.49	17.89	50.62

*Disease scored with 1-9 scale for Alternaria leaf spot was converted to PDI. DAS: Days after sowing,
PDI: Per cent Disease Index

The survey results revealed that the age of the plant could influence the extent and intensity of damage by *A. alternata*. The disease prevalence and severity was less in young and vigorous plants, whereas in old, senescing plants the disease prevalence and severity was more. The amount of damage to sesame plants by *A. sesami* was reported to be dependent on the growth stage of the host (Kolte, 1985) [9].

Uniform severity of Alternaria leaf spot across the villages and mandals may be attributed to variety "LBG 752" which occupied 95% of the area in these surveyed mandals. The data on survey revealed that the little variation in sverity of Alternaria leaf spot observed in the two mandals and the selected villages of each mandal in the present study could be attributed to variation in sowing time of the crop and the differences in adoption of recommended package of practices by the farmers.

Disease symptomology

The typical leaf spot symptoms of *Alternaria* were first appeared on lower leaves at flower initiation stage. The symptoms were initiated from centre as well as margins of the leaves as small (1 to 5 mm in diameter) brown spots that were circular to irregular in shape with definite yellow halo. Later, as the disease progressed, the spots enlarged and fused into

irregular shaped lesions with distinct or indistinct concentric rings. Affected portions in the leaf got separated and fell down resulting in shot holes.

Defoliation occurred on severely affected plants on later stages of the crop growth (Plate1). Symptoms were matched with the description made by Abawi *et al*. (1977) [2], Susuri *et al.* (1982) [12], Kwon *et al*. (2016) [10] and Darai *et al*. (2017) [5].

Fig 1: Typical leaf spot symptoms and progression (A-D) of *A. alternata* on blackgram

Isolation and characterization of the fungus

The fungus was isolated from diseased leaves of blackgram *CV* LBG 752 on PDA and maintained in pure culture.

Cultural characteristics and conidial measurements

Leaf spot sample showing typical symptom was collected separately, surface sterilized and incubated on PDA medium at 27±1 ^{0}C for three days for isolation of the causal organisms. Pure culture of the pathogens was obtained by single spore method and was used for identification and for further investigations. The culture was identified based on the colony characters and conidial dimensions. The fungus *A. alternata* produced initially profuse greyish white mycelium on PDA, later at maturity it became dark brown to black with plenty of conidia (Plate 2).

Fig 2: Pure culture of *A. alternate*

Conidiophores and conidia were golden brown. Conidiophores were simple or branched, straight or curved, 1-3 septate. Conidia were formed individually or in catenulate fashion which were ovoid, obclavate, obpyriform, conical or cylindrical, apical beak not extending one third the length of the conidium, smooth walled or warted, 3-8 transverse septa with one or two longitudinal septa (Plate 3).

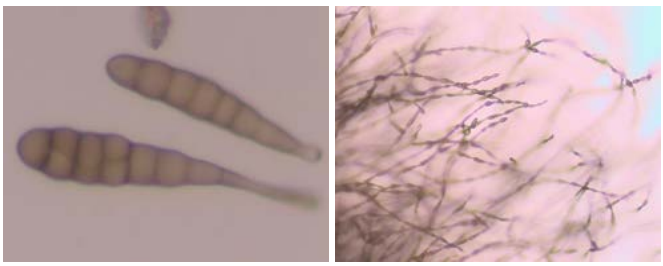

Fig 3: Single Conidium and Catenulate Conidia of *A. Alternata*

These dimensions were subjected to statistical analysis (Table 3). To compare the conidial measurements with earlier reports they were statistically tested with the formula $\mu=\chi \pm t\ 0.05$ (SE) (John, 1970) [7].

Where, μ = Population mean
χ = Sample mean
Standard error (SE) = $\sigma / \sqrt{\eta}$,
σ = Standard deviation
η = Number of spores observed
t = Table t value (P=0.05)

Table 3: Conidial dimensions of *A. alternate*

Characters	Population mean (µm)	Total (µm)	Population mean (µm)	Total (µm)	Range (µm)
Length	53.02 + 1.96 (1.52)	55.99	53.02 - 1.96 (1.52)	50.05	50.05 – 55.99
Breadth	13.61 + 1.96 (0.28)	14.17	13.61 - 1.96 (0.28)	13.06	13.06 – 14.17
Beak length	5.09 + 1.96 (0.20)	5.50	5.09 - 1.96 (0.20)	4.70	4.70 – 5.50

The measurements of the conidia were within the standard ranges (20-63 x 9-18 µm) described by Keissler (1912) [8] and Srinivasan (1994) [11] for *A. alternata.* The colony characters and conidial dimensions of the fungus matched with the standard descriptions for *A. alternata.*

Conclusion

This is the first ever research on spatial distribution of Alternaria leaf spot of blackgram and its characterization in major blackgram growing regions in Guntur district, Andhra Pradesh. This research documented that leaf spot disease is widespread and uniform in blackgram fields. The information generated through this study could help the blackgram growers regarding disease management and selection of resistant cultivars, improving profitability and achieving food security. The outcome of the current endeavor clearly indicated the

prospects of characterization to design the specific diagnostic tools and also to design regional specific management practices.

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