The Pharma Innovation Journal 2022; 11(12): 4215-4218

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 4215-4218

www.thepharmajournal.com
Received: 02-09-2022
Accepted: 09-10-2022

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Pathogenic variability of *Alternaria alternata* isolates from Maharashtra state infecting groundnut crop

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Abstract
Pathogenicity of *A. alternata* 15 isolates was attempted, using *Alternaria* blight susceptible groundnut Local cultivar and spray inoculating the mycelium-cum –spore suspension separately of the test isolates, in screen house clearly indicated that all the 15 isolates of *A. alternata* exhibited a wide range of pathogenic variability. However, the aggressive isolates *viz*., AaDp, AaSg and AaKd were found to be highly virulent, with least incubation period (7 to 8 day), highest lesion frequency (17.00 to 19.00/plant), large sized leaf spots (11.33 to 12.83 mm^2), highest per cent disease incidence (42.33 to 53.29%) and severity (40.42 to 45.17%). Whereas, moderately virulent/aggressive isolates exhibited moderate incubation period (10 to 11 days), moderate lesion frequency (10.00 to 12.89/plant), medium sized spots (4.38 to 9.92 mm^2), moderate disease incidence (16.88 to 34.51%) and disease severity (13.56 to 31.27%); and the least virulent/aggressive isolates exhibited maximum incubation period (13 to 14 days), least lesion frequency (8.50 to 10.66/plant), small sized spots (3.66 to 5.63 mm^2), least disease incidence (12.77 to 18.49%) and disease severity (13.45 to 15.33%).

Keywords: Pathogenic variability, *Alternaria alternata*, infecting groundnut crop

Introduction
Groundnut (*Arachis hypogea* L.) is one of the most important leguminous oilseeds crop belonging to family fabaceae and sub-family Papilionaceae, which comprise important edible oilseed crops in the World. The major groundnut producing countries in the world are India, China, Nigeria, Senegal, Sudan, Burma and the United States of America. The major fungal disease infecting groundnut are stem rot (*Sclerotium rolfsii* Sacc.), wilt (*Fusarium oxysporum*), pod rot (*Rhizoctonia solani*), rust (*Puccinia arachidis*), early leaf spot (*Cercospora arachidicola*), late leaf spot (*Cercosporidium personatum*) and leaf blight (*Alternaria alternata*); bacterial wilt (*Pseudomonas solanacearum*) and viral bud necrosis (TSWV), clump, rosette, peanut strip and mottle (Subrahmanyam *et al*., 1981) [9]. Leaf blight caused by *Alternaria alternata* is one of the most widely distributed and destructive disease of groundnut, causing about 13-22 per cent yield losses.

Materials and Methods
Pathogenicity and pathogenic variability of *A. alternata* isolates
In order to establish host-pathogen interaction and confirm cause of the disease, pathogenicity test for all 15 isolates of *A. alternata* was attempted in black polythene bags, under screen house conditions. Seeds of groundnut Local cultivar susceptible to *Alternaria* blight (*A. alternata*) were surface sterilized with 1-2% Sodium hypochloride (NaOCl) solution for 1-2 min. and sown (@ 10 seeds/bag) in the polythene bags (20 × 30 cm) filled with chemically sterilized (4-5% solution of formalin) potting mixture (3 parts soil + 1 part Fym.).
The spore-cum-mycelial suspensions of *A. alternata* each test isolate were prepared separately from 15 days old pure culture in plates, by flooding with 10 ml sterile distilled water. This resultant suspension was suitably diluted with sterile distilled water to obtain inoculum concentration of 2 x 10^6 spores/ml.
Twenty one days old seedlings of groundnut Local cultivar growing in polythene bags were artificially spray inoculated separately with spore-cum- mycelial suspension of the test isolates. For each test isolate, 30 groundnut seedlings were spray inoculated. Groundnut Local cultivar seedlings grown in polythene bags and sprayed with sterile water (without inoculum) were maintained as un-inoculated control. These polythene bags (both inoculated and un-inoculated) were covered with polythene bags during evening hours and kept overnight, watered regularly to create optimum relative humidity and maintained in screen house for development of the disease symptoms.

To assess pathogenic variability among the test isolates, the observation on various parameters *viz*., incubation period, size and frequency of the spots, per cent disease incidence and severity were recorded. The disease severity was recorded by applying following 0 to 9 grade disease rating scale (Kumar *et al*., 2012) [3].

Scale/Grade	Descriptions
1	0% blighted leaf area
2	1-5% blighted leaf area
3	6-10% blighted leaf area
4	11-20% blighted leaf area
5	21-30% blighted leaf area
6	31-40% blighted leaf area
7	41-60% blighted leaf area
8	61-80% blighted leaf area
9	Above 80% blighted leaf area

Per cent Disease Severity was calculated by applying the following formula (Wheeler, 1969) [12].

$$\text{Per cent Disease Severity} = \frac{\text{Sum of individual numerical ratings}}{\text{Total number of leaves observed x Maximum disease grade}} \text{x } 100$$

Considering, per cent disease severity, the test isolates were categorized as highly virulent, moderately virulent, least virulent and avirulent, if any.

Based on pathogenicity traits and disease severity, the most virulent isolate found was AaDp (Dapoli), which was promoted for further *in vitro* studies.

From the artificially inoculated and diseased groundnut leaves, the test pathogen isolates were reisolated separately on PDA medium and incubated at 28±2 °C. After a week of incubation the cultural and morphological characteristics developed of the test isolates were observed and compared with the characteristics (cultural and morphological) of the original test isolates obtained from naturally *Alternaria* blight diseased groundnut foliage. To satisfy Koch's postulates, symptoms developed on artificial inoculated groundnut leaves were compared with original symptoms on naturally diseased plants.

Table 1: Pathogenicity traits and Pathogenic variability among the test isolates of *A. alternata*

Isolates	IP (days)*	Lesion Frequency/ Plant*	Av. Size of Spot (mm^2)*	PDI (%)	PDS (%)	VG
AaLt	11	11.33	6.00	19.64 (26.30)	17.41 (24.66)	MV
AaDp	07	19.00	12.83	53.29 (46.88)	45.17 (42.22)	HV
AaSg	08	17.50	11.67	44.86 (42.04)	41.87 (40.32)	HV
AaKd	08	17.00	11.33	42.33 (40.58)	40.12 (39.30)	HV
AaVh	10	12.18	8.45	29.78 (33.07)	27.67 (31.73)	MV
AaPt	10	12.89	9.92	34.51 (35.97)	31.27 (34.00)	MV
AaPb	11	10.50	5.41	18.03 (25.12)	15.18 (22.93)	MV
AaNd	11	10.16	5.19	17.23 (24.52)	14.58(22.44)	MV
AaAk	13	10.00	4.83	16.88 (24.25)	14.40(22.30)	MV
AaHn	11	11.16	5.57	19.11 (25.92)	16.66 (24.08)	MV
AaWs	13	10.66	5.63	18.49 (25.46)	15.33 (23.05)	LV
AaAn	10	12.33	8.16	29.96 (33.18)	28.09 (32.00)	MV
AaSl	12	9.00	4.38	16.43 (23.91)	13.56 (21.60)	MV
AaKr	10	12.48	8.50	30.33 (33.41)	28.83 (32.47)	MV
AaPn	14	8.50	3.66	12.77 (20.93)	13.45 (21.51)	LV
S.E. ±	0.68	0.77	0.63	0.57	0.45	------
C.D.(P =0.01)	1.98	2.23	1.83	1.67	1.31	------

*: Mean of three replications, PDI: Per cent disease incidence, PDS: Per cent disease severity, Avirulent (AV) = No disease, Least virulent (LV) = 1.00-20.00% disease severity, Moderately (MV) = 21.00-40.00% disease severity, highly virulent (HV) = > 40% disease severity, IP – Incubation period, Av.: Average, No. Number, VG- Virulence grade, Lesion frequency (Av. no. of spots / plant).

Results and Discussion

Pathogenicity and pathogenic variability of *A. alternata* isolates

Pathogenicity of *A. alternata* 15 isolates was attempted, using *Alternaria* blight susceptible groundnut Local cultivar and spray inoculating the mycelium-cum –spore suspension separately of the test isolates, in screen house (Plate 1).The results obtained on pathogenic traits *viz*., incubation period, lesion frequency and its size, per cent disease incidence, disease severity and isolate virulence pattern / grading, are presented in Table 1.

Symptoms

All 15 test isolates of *A. alternata* were found pathogenic to groundnut. The symptoms induced were identical to those symptoms observed on naturally diseased groundnut crop foliage (Plate 2).

Incubation period

Results (Table 1) revealed that among the isolates, incubation period varied from 7 (AaDp) to 14 (AaPn) days. However, It was least (7 to 8 days) with the isolates *viz*., AaDp, AaSg and AaKd; moderate (10 to 11 days) with the isolates *viz*., AaVh, AaPt. AaLt, AaKr, AaAn, AaPb, AaNd and AaHn and maximum (> 12 days) with the isolates *viz*., AaSl, AaAk, AaWs and AaPn.

Lesion frequency (Av. no. of spots/plant)

The results (Table 1) revealed that among the isolates induced lesion frequency on artificially inoculated / diseased groundnut seedlings, ranged from 8.50 (AaPn) to 19.00 (AaDp). However, it was minimum (8.50 to 11.00) with the isolates AaPn (8.50), AaSl (9.00), AaAk (10.00), AaNd (10.16), AaPb (10.50) and AaWs (10.66); moderate (11.1to 13.00) with the isolates *viz.*, AaHn (11.16), AaLt (11.33), AaVh (12.18), AaAn (12.33), AaKr (12.48) and AaPt (12.89) and maximum (> 13.1) with the isolates *viz.*,AaKd (17.00), AaSg (17.50) and AaDp (19.00).

Lesion / spot size

Results (Table 1) revealed that among the isolates, lesion size varied from 3.66 (AaPn) to 19.00 mm^2 (AaDp). However, It

was small (3 to 6 mm^2) with the isolates AaPn (3.66 mm^2), AaSl (4.38 mm^2), AaAk (4.83 mm^2), AaNd (5.19 mm^2), AaPb (5.41 mm^2), AaHn (5.57 mm^2), AaWs (5.63 mm^2) and AaLt (6.00 mm^2); medium (6.1 to 10.00 mm^2) with the isolates *viz*., AaAn (8.16 mm^2), AaVh (8.45 mm) AaKr (8.50 mm) and AaPt (9.92 mm^2), and Large (> 10 mm^2) with the isolates *viz*., AaKd (11.33 mm^2), AaSg (11.67 mm^2) and AaDp (12.83 mm^2).

Per cent disease incidence

Results (Table 1) revealed that the per cent disease incidence induced by the test isolates, varied from 12.77% (AaPn) to 53.29% (AaDp). However, it was least (12 to 20%) with the isolates *viz*., AaPn (12.77%), AaSl (16.43%), AaAk (16.88%), AaNd (17.23%), AaPb (18.03%), AaWs (18.49%), AaHn (19.11%) and AaLt (19.64%); moderate (20.1 to 40.00%) with the isolates *viz*., AaVh (29.78%), AaAn (29.96%), AaKr (30.33%) and AaPt (34.51%) and highest (> 40.00%) with the isolates *viz*., AaKd (42.33%), AaSg (44.86%) and AaDp (53.29%).

Per cent disease severity

Results (Table 1) revealed that the per cent disease severity induced by the test isolates varied from 13.45% (AaPn) to 45.17% (AaDp). However, it was least (12 to 20%) with the isolates *viz*., AaPn (13.45%), AaSl (13.56%), AaAk (14.40%), AaNd (14.58%), AaPb (15.18%), AaWs (15.33%), AaHn (16.66%) and AaLt (17.41%); moderate (20.1 to 40.00%) with the isolates *viz*., AaVh (27.67%), AaAn (28.09%), AaKr (28.83%) and AaPt (31.27%) and highest (> 40.00%) with the isolates *viz*., AaKd (40.12%), AaSg (41.87%) and AaDp (45.17%).

(A) **(B)**

Plate 1: Pathogenicity (A) and pathogenic variability (B) of the test isolates of *A. alternata*

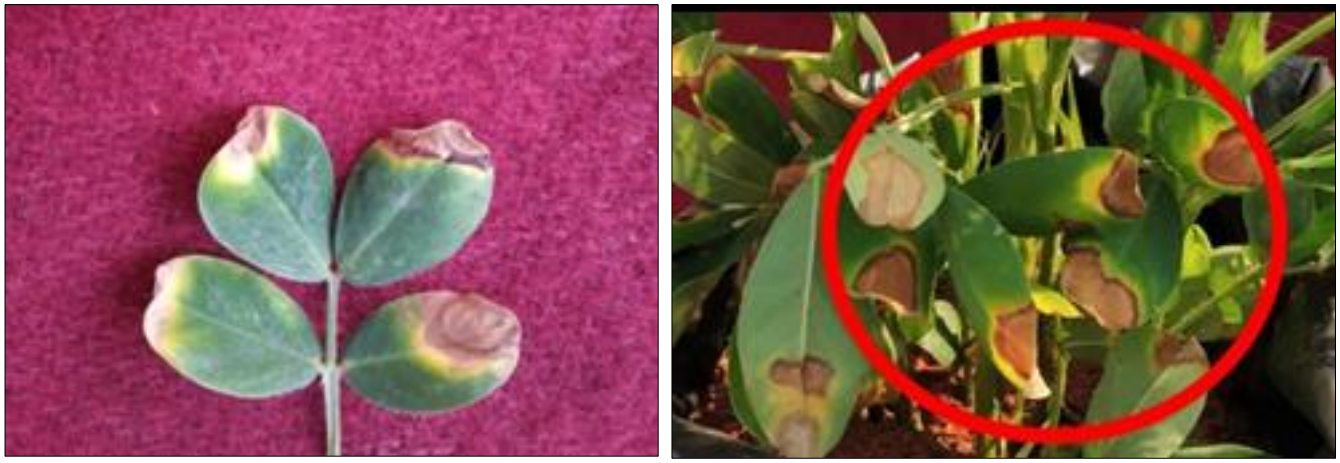

Plate 2: Symptomatology of groundnut *Alternaria* leaf blight

Virulence pattern / grading of the test isolates

By considering incubation period, lesion frequency and size and per cent disease severity, the virulence pattern / grading of *A. alternata* test isolates was assessed, by applying the virulence grading scale: Avirulent (AV) = No disease, Least virulent (LV) = 1.00 to 20.00% disease severity, maximum incubation period and minimum lesion frequency; Moderately virulent (MV) = 21.00 to 40.00% disease severity, moderate incubation period, moderate lesion frequency and Highly virulent (HV) = > 40% disease severity, least incubation period and maximum lesion frequency.

The results (Tabel 1) revealed three isolates, *viz*., AaDp, AaSg and AaKd as highly virulent with > 40 per cent disease severity, ten isolates *viz.*, AaLt, AaVh, AaPt, AaPb, AaNd, AaAk, AaHn, AaAn, AaSl and AaKr as moderately virulent with 21.00 to 40.00 per cent disease severity and two isolates *viz.*, AaWs and AaPn as least virulent with 1.00 to 10.00 per cent disease severity.

Based on pathogenicity traits and virulence pattern / grade, the isolate AaDp was promoted for further various *in vitro* studies.

Thus, pathogenicity as well as pathogenic variability of all 15 isolates of *A. alternata*, causing groundnut leaf blight / spot disease was attempted and proved successfully.

Pathogenic association of *A. alternata* with groundnut, causing *Alternaria* leaf spot / blight was reported earlier by

several workers under controlled conditions and successfully proved its pathogenicity (Kumar *et al*., 2012; Kantwa *et al*., 2014; Reddy, 2018; Zhang *et al*., 2021) [3, 1, 6, 13].

In the present study, among 15 isolates of *A. alternata* (groundnut leaf blight), we found the isolate AaDp (Dapoli) as most virulent, with least incubation period (7 days), maximum lesion frequency and size (19.00 and 12.83mm^2) and highest disease severity (45.17%). This was followed by the isolates *viz*., AaSg and AaKd.

Pathogenic/virulence variability of a particular fungal pathogen/isolate may be attributed to several factors, such as plant host susceptibility/resistance, weather variables, locality/regions, production of cellulolytic and pectinolytic enzymes, physiological parameters, ability to produce toxins, hosts structural and anatomical barriers etc. (Sharma *et al*., 2013) [8].

Pathogenic diversity among the isolates of various *Alternaria* spp. infecting different oilseeds and other crops was reported previously by several researchers (Verma *et al*., 2007; Kumar *et al*., 2008; Rajender *et al.*, 2013; Saha *et al*., 2015; Nikam *et al*., 2015; Wagh *et al.*, 2020) [10, 2, 5, 7, 4, 11].

Considering, various pathogenicity traits especially, incubation period, lesion frequency and per cent disease severity, the earlier workers who reported pathogenic variability in various *Alternaria* spp. were: Verma *et al*. (2007) [10] reported variability among the isolates of *A. solani* (tomato early blight), Kumar *et al*. (2008) [2] reported variability among the isolates of *A.solani* (tomato early blight), Rajender *et al.*(2013) [5] reported variability among the isolates of *A. helianthi* (sunflower leaf blight),Saha *et al*. (2015) reported variability among the isolates of *A. brassicae* (cauliflower leaf blight), Nikam *et al*. (2015) [4] reported variability among the isolates of *A. solani* (tomato early blight) and Wagh *et al.* (2020) [11] reported variability among the isolates of *A. carthami* (safflower leaf blight).

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