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# Studies on *Cercospora* leaf spots disease of groundnut under Bundelkhand region

# Adesh Kumar, Abhijeet Srivastava, Nishi Roy, Arjun Verma and Hitesh Kumar Saini

#### Abstract

Tikka disease of groundnut caused by *Cercospora* spp. has economic importance to reduced crop yield. Twenty two isolate of *Cercospora* spp. *were* recovered from diseased leaves and clustered using UPGMA on the basis of disease reaction on four cultivars (TG-37A, Kadiri-6, Kaushal and Junku). It was observed that three isolates namely C10, C11 and C12 were highly virulent on almost all the groundnut cultivars. Junku variety allowed highest low virulent isolates per cent 77.27 and this variety showed moderate resistance reaction to comparatively less susceptible to Jhansi population of *Cercospora* spp. The *in vitro* and *in vivo* antifungal effects of the eleven fungicides were investigated. Under *in vitro*, all the fungicides showed most toxic reaction with 50% effective concentrations (EC<sub>50</sub>) of < 1 ppm. However, they varied in EC<sub>90</sub> values. Twenty ppm concentrations of Amistar Top (Strobilurin+Azole) showed 90% effective concentration (EC<sub>90</sub>). Amistar (Strobilurin) also showed approximately same results as Amistar Top while EC<sub>90</sub> value of Natio, Score, Tilt, Lusture, Folicur, Contaf and Kavach was <25ppm. While, SAAF and Carbendazim showed less toxicity as compared other fungicides with EC<sub>90</sub> value at concentrations >50 ppm. It was also observed Strobilurin+Azole group fungicides also dominant in the field condition to control the disease.

Keywords: Tikka disease, cultivars, pathogenicity, groundnut, virulence, Cercospora spp. and fungicides

#### Introduction

Groundnut (Arachis hypogaea) is major edible oilseed crop of India. It is fourth most important source of edible oil and third most important source of vegetable protein. Globally, the crop is raised on 26.4 million hectares with a total production of 37.1 million MT with average productivity is 1400 kg/ha (Anon 2017)<sup>[1]</sup>. In kharif-2017, the all-India acreage was 41, 52, 500 hectares. Eight states viz., Gujarat, Andhra Pradesh, Rajasthan, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu and Uttar Pradesh were identified to have acreages greater than one lakh hectares and these states jointly accounted for 95.5% of the national acreage (Anon 2017)<sup>[1]</sup>. The area under groundnut in Uttar Pradesh was 115000 hectares during Kharif 2017 and rainfed area of Bundekhund also play important role in groundnut production. Groundnut production is hampered by a number of abiotic and biotic factors such as unfavorable climate, nutritional imbalance, diseases caused by fungi and bacteria. Among the biotic factors, the diseases caused by fungi are economically more important because they cause heavy yield losses. Tikka and rust both fungal disease caused losses up to 70% (McDonald et al., 1985; Miller et al., 1990; Grichar et al., 1998) <sup>[7, 9, 3]</sup>. resulting in a lower productivity of the crop. Tikka disease alone caused 10 to 50% yield losses and without application of fungicides losses would be increases however, losses depend on locality, season and climatic condition (McDonald et al. 1985)<sup>[7]</sup>. Tikka disease of groundnut is caused by two species of Cercospora: Cercospora personata (Berk. & Curt.) Elle and Eve, now known as Cercosporidium personatum (Berk. & Curt.) Deighton and Cercospora arachidicola Hori. All aerial parts of the plant are infected by the pathogen. The groundnut leaf spots (early leaf spot and late leaf spot) commonly called as "Tikka" disease cause nearly complete defoliation and yield loss up to 50 per cent or more depending upon disease severity. The first visible symptoms appear on the leaflets of lower leaves as dark spots which at a later stage, are surrounded by yellow rings. Spots due to C. personata are more circular and smaller than those produced by C. arachidicola. These are the typical 'Tikka' disease spots which almost cover the entire leaf surface in an epiphytotic condition of the disease. In Bundelkhund,

invariably appears every year in the groundnut field causing significant yield and quality loss. this disease Realizing the importance of this disease, the present studies were initiated to isolate, identify and characterize the pathogen with respect to its aggressiveness and also to evaluate different fungicides in vitro and in vivo against this pathogen.

#### **Method and Material**

#### **Collection of diseased samples**

Different groundnut growing areas of Jhansi district, Uttar Pradesh come under Bundelkhund region were surveyed in the months of July-August during kharif 2018. During this survey 6 blocks of the district were covered viz; Babina. Bdagaon, Bangra, Gursaray, Chirgaon and mauranipur. At each location, disease incidence and severity was recorded, the infected diseased samples were put in plastic bags and kept on ice. The samples were stored 4°C before further processing.

#### Isolation of the pathogen

Diseased leaves were collected from 6 blocks of the Jhansi district in the month of July- August, 2018 (Figs. 1A and B). After surface sterilization, the leaf pieces were placed on potato dextrose agar separately and grown at 25 °C for 7-10 days. The rising fungal hyphal tips were transferred to PDA and grown for 14-15 days, and cultures were further purified by single spore isolation method. The morphological characteristics of the fungi were analyzed using microscope at 100X. Twenty two isolates of the Tikka disease pathogen were recovered and used for this study.

#### Pathological characterization and aggressiveness

Four groundnut varieties viz. TG-37A, Kadiri-6, Kaushal and Junku procured from Krishi Vigyan Kendra, Bharari Jhansi, were grown on 2st week of July 2018 in the field of Krishi Vigyan Kendra, Bharari Jhansi, using standard package of practices (Anon 2018). Individual cultivars were grown on flat beds in 4 meter long rows with plant to plant distance of 10 cm. seven rows of each cultivar were raised at a spacing of 30 cm. One month old plants of individual cultivars were inoculated with all the 22 isolates of *Cercospora* spp. Twenty days mycelium of all tested isolates was harvested from the petriplates with distilled water and diluted five times and inoculated with spray method. After inoculation moisture was maintained with artificial method. The plants were frequently irrigated to keep up high humidity and soil moisture which is vital for disease development. The disease symptoms were observed with varying day's interval. Pathogen virulence efficiency was recorded on the basis of 1-9 disease scale (Subbarao et al. 1990)<sup>[15]</sup>. (Table-1). The pathogen was reisolated from the infected inoculated fruits and reconfirmed as *Cercospora* spp. Initial disease symptoms were observed after 16 days of inoculation and final observations were recorded after 30 days. Data was analysed with help of PAST ver 2.5.1 software.

Table 1: Disease scale 1-9 (Subbarao et al. 1990)<sup>[15]</sup>.

Scale	Description
0	No disease
1-5	Lesions present largely on lower leaves; no defoliation.
6-10	Lesions present largely on lower leaves; very few lesions on middle leaves; defoliation of some leaflets evident on lower leaves.
11-20	Lesions are present on lower and middle leaves but severe on lower leaves; defoliation of some leaflets evident on lower leaves.
21-30	Lesions are present on all lower and middle leaves; over 50% defoliation of lower leaves.
31.40	Lesions severe on lower and middle leaves; lesions present on Top leaves but less severe; extensive defoliation of lower leaves;
51-40	defoliation of some leaflets, evident on middle leaves.
40-60	Lesions present on all leaves but less severe on top leaves; defoliation of all lower and some middle leaves.
61-80	Defoliation of all lower and middle leaves; lesions severe on top leaves and some defoliation of top leaves evident.
80-100	Defoliation of almost all leaves leaving bear stems; some leaflets may be present but with severe leaf spots.

#### In vitro efficacy of different fungicides against Cercospora spp.

Fungicides (Table-2) were tested to determine their 50% effective concentration (EC50) and EC90 values for the inhibition of mycelial growth. To investigate the inhibition of mycelial growth of Cercospora spp. five replicate PDA plates (90 mm in diameter) containing the fungicides were prepared at concentrations of 1, 10, 20, 25 and 50ppm. The control plates contained only PDA medium. Individual agar disks (6

mm in diameter) were removed from the edge of an actively growing culture (C12) and placed at PDA plates that containing fungicides and incubated at 25 °C for 10-15 days. The mycelium growth of pathogen was measured by scale and compared with control growth. The concentration of each fungicide causing 50% (EC<sub>50</sub>) or 90% (EC<sub>90</sub>) reduction in mycelial growth compared to the absence of the fungicide was estimated referring to Matheron and Porchas (2000) and based on the estimated values.

Fungicides	Chemical group	Active ingredient	Concentration (%)	Formulation
Folicur	Azole	Tebuconazole	25.9	EC
Tilt	Azole	Propiconazole	25	EC
Natio	Mixture of Azole and Strobilurin	Tebuconazole+ Trifloxystrobin	50+25	WG
SAAF	Mixture of benzimidazole and Carbamate	Carbendazim+ Mancozeb	12+63	WP
Bavistin	Benzimidazole	Carbendazim	50	WP
Amistar	Strobilurin	Azoxystrobin	23.0	W/W
Amistar Top	Strobilurin+Azole	Azoxystrobin+ Difenoconazole	18.2+11.4	W/W
Score	Azole Difenoconazole		250	EC
Contaf	Azole	Hexaconazole	5	EC
Lusture	Azole+ Benzimidazole	Flusilazole+ Carbendazim	12.5+25	SE
Kavach	Chloronitrile	Chlorothalonil	78.12	WP

Table 2: Fungicides	used	in	this	study
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The field experiment was conducted at Ghandinagar village (NICRA village) come under Badagaon block of district Jhansi in the month of June-September, 2018. Groundnut cultivar Kadiri-6 was grown in experiment field area with plot size 4×3 square meters. The virulent isolate of Cercospora spp. (C12) was inoculated one month old plants with spore suspension spray method. Disease incidence and severity was observed after 20 days of inoculation using 1-9 scale (Subbarao et al. (1990)<sup>[15]</sup>. Twelve treatments were applied in this experiment viz; T1-Folicur@1ml/lit, T-2 Tilt@1ml/lit, T-3 Natio@1gram/lit, T4-SAAF@2gram/lit, T5-Carbendazim @2gram/lit, T-6 Amistar@1ml/lit, T-7 Amistar Top @1ml/lit, T-8Score@1ml/lit. Contaf@1ml/lit. T-9 T-10 Lusture@1ml/lit, T-11 Kavach@2gram/lit and T12 control (no chemical used).

#### **Statistical Analysis**

The pathotypic similarity between the various isolates was generated using unweighted paired group mean averages using software programme PAST ver. 2.1.5.

#### **Result and Discussion**

Tikka disease is an important constraint in the cultivation of kharif groundnut. Groundnut is mainly grown in the six blocks of district Jhansi namely; Babina. Bdagaon, Bangra, Gursaray, Chirgaon and mauranipur. The village wise surveys conducted during 2018 Kharif cropping seasons revealed that the disease infestation was invariably present in all surveyed villages (Table-3). A total of 20 villages were visited come under six blocks of district Jhansi. It was observed that maximum Tikka disease incidence and severity was observed in villages Gandhinagar (70% and 48%) come under Badagaon block and second highest disease incidence and severity was observed in village Birgua with 65% and 46% respectively. However, at other locations disease incidence and severity was observed to be low. It also observed that village Tejpura come under block Bangra showed lowest disease incidence and severity percent 22 and 12 respectively.

S. No.	Village	Block	District	DI	DS
1	Ghandinagar	Badagaon	Jhansi	70	48
2	Birgua	Badagaon	Jhansi	65	46
3	Lakara	Badagaon	Jhansi	45	32
4	Keshavpur	Badagaon	Jhansi	62	42
5	Bhojla	Badagaon	Jhansi	52	44
6	Behta	Badagaon	Jhansi	60	46
7	Baltada	Badagaon	Jhansi	54	48
8	Behta	Badagaon	Jhansi	52	40
9	Booda	Badagaon	Jhansi	58	52
10	Kot	Badagaon	Jhansi	50	38
11	Dhikauli	Babina	Jhansi	58	40
12	Sinthari	Chirgaon	Jhansi	58	46
13	Bdawali	Bangra	Jhansi	52	25
14	Rajpura	Bangra	Jhansi	44	36
15	Palra	Bangra	Jhansi	44	22
16	Kachwada	Bangra	Jhansi	28	24
17	Pathha	Bangra	Jhansi	24	18
18	Tejpura	Bangra	Jhansi	22	12
19	Chokri	Bangra	Jhansi	28	16
20	Divyapur	Moth	Jhansi	24	14

Table 3: Survey for Tikka disease of groundnut in different blocks of Jhansi district.

#### Isolation and identification of the pathogen

Twenty two isolates were recovered from infected leaf samples (Plate-1). All the recovered isolates were showed specific morphological characters of *Cercospora* spp. The culture of *Cercospora* spp. initially showed white colonies on Potato dextrose agar (PDA) at  $25 \pm 2^{\circ}$ C. The mycelium was septate and produced conidiophores. Conidiophores are long, smooth and Conidia were obclavate to cylindrical, light coloured, 1-7 septate with bluntly rounded ends (Singh 1998).



А

Plate 1: (A) Disease infected levease and (B) Cercospora sp. fungi growth

#### Pathological characterization and aggressiveness

Response of different isolates with respect to aggressiveness was studied. The aggressive of the isolates were measured on the based disease severity scale 1-9. Nine days after inoculation, the symptoms started appearing on inoculated leaves. In the beginning, the small chlorotic spots were noticed and later spots enlarged. The symptoms observed on the inoculated plants were typical to the symptoms that were observed in the field conditions. Many authors also widely acknowledged about Tikka disease of symptom caused by C. arachidicola and C. personatum, (Mcdonald et al. 1985; Miller et al. 1990; Grichar et al. 1998) [7, 9, 3]. The data presented in Fig. 1 shows the presence of significantly diverse virulence spectrum in Cercospora spp. populations of Jhansi dirstrict come under Bundelkhand region of Uttar Pradesh. Disease response with respect to 22 isolates of Cercospora spp. clustered using UPGMA (Fig. 1). The data plotted in dendrogram (Fig. 1) showed three isolate viz. C10, C11 and C12 to be clustered together and were classified in group A

(table-4).These isolates were found to be highly virulent on almost all the cultivars tested producing highest disease mean disease score 8 and range 7 to 9. Eight isolates namely C5, C13, C14, C15, C16, C20, C21 and C22 fall under group B-1 and secured mean disease score 4.15 with range 4- 4.5. Remaining eleven isolates namely C1, C2, C3, C4, C6, C7, C8, C9, C17, C18 and C19 come under group B1 and secured mean disease score 2.79 (table-4).



Fig 1: Clustering of different isolates of *Cercospora sp.* on the basis of disease severity on four cultivars of groundnut

 Table 4: Grouping of 22 isolates of *Cercospora sp.* on the basis of virulence on four groundnut cultivars.

Group	Name of Isolates	No. of	Incul Period	bation l (days)	Remark	
_		isolates	Mean	Range		
А	C10, C11 and C12	3	8.0	7.0-9.0	Highly Virulent (HV)	
B-1	C5, C13, C14, C15, C16, C20, C21 and C22	8	4.15	4-4.5	Moderately Virulent (MV)	
B-2	C1, C2, C3, C4, C6, C7, C8, C9, C17, C18 and C19	11	2.79	2-3.25	Less Virulent (LV)	

Virulence frequency of *Cercospora* spp. isolates on four Groundnut cultivars

In the present study four groundnut cultivars were screened (Fig. 2) which showed different reaction to Jhansi population of Cercospora spp. Kadiri variety of groundnut allowed 18.18% isolates for severe infection and 68.18% isolates showed moderate virulent reaction on this variety. However, another three variety namely TG-37A, Kaushal and Junku allowed only 13.63% isolates to caused severe infection and showed highly virulent reaction but moderate virulent isolates % was less in Junku variety as compared to all varieties. Junku variety showed highest low virulent isolates per cent 77.27 and this variety showed moderate resistance reaction to comparatively less susceptible to Jhansi population of *Cercospora* spp. Similarly, Subrahmanyam *et al.* (1983) <sup>[16]</sup>. Screened 23 groundnut germplasm lines against rust and tikka diseases. Among these, nine were resistant to P. arachidis and six of these were also resistant to *M. berkeleyii*. Gupta (1987) also reported that only 21 of 253 groundnut cultivars screened in the field were resistant to C. arachidicola and C. personatum. Many others authors also screened groundnut germ plasm against C. arachidicola and C. personatum (Chaudhary 1988; Mehan et al. 1996; Moss et al., 1997; Pensuk et al. 2003; Jyosthna et al. 2004; Hossain et al. 2007) [2, 8, 10, 13, 5, 4]



Fig 2: Virulence frequency of 22 isolates of *Cercospora sp.* on four groundnut cultivars.

# *In vitro* efficacy of different fungicides against *Cercospora* spp.

The study revealed the efficacy of eleven fungicides viz: T1-Folicur@1ml/lit, T-2 Tilt@1ml/lit, T-3 Natio@1gram/lit, T4-SAAF@2gram/lit, T5-Carbendazim @2gram/lit, T-6 T-7 Amistar @1ml/lit. Amistar@1ml/lit, Top T-8 Score@1ml/lit, T-9 Contaf@1ml/lit, T-10 Lusture@1ml/lit, T-11 Kavach@2gram/lit against pathogen (Table 5 and 6). The EC<sub>50</sub>/EC<sub>90</sub> values of all fungicides against Cercospora spp. were observed (Table 5 and 6). The  $EC_{50}$  values of Cercospora spp. for the all fungicides were <1ppm, these reduced the fungi growth more than half at this concentration. Strobilurin combination with Azole group (Amistar Top) and alone fungicides (Amistar) were inhibited the mycelial growth of Cercospora spp most effectively even fungi not able to start growth at concentration of 25 ppm com-pared to the others fungicides viz. Folicur, Tilt, Natio, SAAF, Bavistin, Score, Contaf, Lusture and Kavach. However, Twenty ppm concentrations of Amistar Top (Strobilurin+Azole) showed 90% effective concentration (EC<sub>90</sub>). Amistar (Strobilurin) also showed approximately same results as Amistar Top while EC<sub>90</sub> value of Natio, Score, Tilt, Lusture, Folicur, Contaf and Kavach was <25ppm. While, SAAF and Carbendazim showed less toxicity as compared other fungicides with EC<sub>90</sub> value at concentrations >50 ppm. Present study revealed that Strobilurin combination with Azole group (Amistar Top) and

alone fungicides (Amistar) were more effective to inhibiting the mycelial growth of *Cercospora* spp. than other fungicides

based on EC<sub>50</sub> and EC<sub>90</sub> values (Table 5 and 6).

	Mycelial growth (mm)							
Fungicides	Concentration in ppm							
	1	5	10	20	25	50		
Folicur	22.4 (.22)	20.4 (.24)	16.2 (.52)	11.8 (.22)	5.20 (.23)	0		
Tilt	22.8 (.53)	17.2 (.26)	12.0 (.23)	10.5 (.93)	4.20	0		
Natio	19.18 (.13)	16.38 (.22)	15.4 (.28)	12.8 (.23)	4.0 (.20)	0		
SAAF	41.8 (.32)	23.4 (.40)	18.4 (.43)	13 (.29)	9.2 (.53)	4.6 (.83)		
Carbendazim	28.4 (.22)	19.6 (.22)	17.2 (.73)	15.4 (.28)	12.8 (.23)	5.0 (.20)		
Amistar	19.2 (.13)	11.4 (.22)	9.5 (.12)	4.5	0	0		
Amistar Top	19.2 (.13)	11.4 (.22)	9.5 (.12)	5.6 (.22)	0	0		
Score	20.4 (.23)	18.4 (.24)	16.2 (.53)	11.8 (.23)	5.8 (.20)	0		
Contaf	28.6 (.22)	19.8 (.22)	17.2 (.12)	16.4 (.28)	8.8 (.23)	0		
Lusture	30.4 (.22)	28.6 (.22)	17.2 (.62)	16.4 (.28)	5.8 (.20)	0		
Kavach	38.4 (.24)	32.12 (.26)	27 (.12)	15.4 (.20)	8.90 (.14)	0		
Control								

 Table 5: In vitro efficacy of different fungicides against Cercospora spp.

Figures in parentheses are standard deviations from mean of five replications

Table 6: EC50 and	EC90	values	of my	celial	growth	of Cerc	cospora
	spp.	for the	e six fu	ingicio	des		

Fungicides	EC <sub>50</sub>	EC <sub>90</sub>
Folicur	<1ppm	<25ppm
Tilt	<1ppm	<25ppm
Natio	<1ppm	<25ppm
SAAF	<1ppm	<50ppm
Carbendazim	<1ppm	<50ppm
Amistar	<1ppm	<20ppm
Amistar Top	<1ppm	<20ppm
Score	<1ppm	<25ppm
Contaf	<1ppm	<50ppm
Lusture	<1ppm	<25ppm
Kavach	<1ppm	<50ppm

### Efficacy of different fungicides against *Cercospora* spp. under field Condition

All the fungicides play important role in disease incidence and severity reduction per cent under field condition (Table-7, fig-3) However Strobilurin with Azole group (Amistar Top)

showed dominant to reduce the tikka disease incidence and severity. It was observe that disease reduction per cent of Amistar Top higher which reduced 91.25% disease incidence and 88.90% disease severity. Ninety per cent disease incidence reduction per cent of three fungicides (Amistar, Natio and Lusture) was observed with little difference disease severity. Tilt, Score, Folicur, SAAF, Bavistin and Contaf also showed good result with respect to disease incidence reduction (88.50, 87.50, 87.50, 87.50, 85 and 82.50%) and severity reduction (85.20, 78.90 82.20, 83.30, 80, 77.8%) respectively. The trails were revealed that application of Strobilurin with Azole group (Amistar Top) was very effective in controlling the disease (Table 7 and Fig. 3). Khan et al. (2014)<sup>[6]</sup>. also found combination of Strobilurin and Azole group of fungicides reduced maximum disease incidence and severity. Mushrif et al. (2017)<sup>[11]</sup>. also reported that the tebuconazole (0.1 per cent) was effective in registering least disease severity in terms of percent disease intensity. Many authors also found similar results (Tiwari et al. 2004; Vemana et al. 2005; Nutsugah et al. 2007)<sup>[17, 18, 12]</sup>.

**Table 7:** Chemicals used under field condition with their concentration

Treatments	Chemicals	Chemical dose under field condition	DI	DS	<b>DI Reduction %</b>	DS Reduction %
T1	Folicur	0.1%	16	10	82.22	87.5
T2	Tilt	0.1%	13	9	85.56	88.75
T3	Natio	0.1%	14	8	84.44	90
T4	SAAF	0.2%	15	10	83.33	87.5
T5	Bavistin	0.2%	18	12	80.00	85
T6	Amistar	0.1%	12	8	86.67	90
T7	Amistar Top	0.1%	10	7	88.89	91.25
T8	Score	0.1%	19	10	78.89	87.5
Т9	Contaf	0.1%	20	14	77.78	82.5
T10	Lusture	0.1%	12	8	86.67	90
T11	Kavach	0.2%	18	14	80.00	82.5
T12	Control		90	80	0.00	0



Fig 3: Effect of different chemicals on percent reduction of Tikka disease incidence (DI) and disease Severity (DS) in groundnut

#### Conclusion

Twenty two isolates of *Cercospora* spp. were recovered from diseased leaves collected different blocks of district Jhansi. Three isolates namely C10, C11 and C12 were highly virulent on almost all the groundnut cultivars and Junku variety showed moderate resistance reaction to comparatively less susceptible to Jhansi population of *Cercospora* spp. Under *in vitro*, all the fungicides showed most toxic reaction with 50% effective concentrations (EC<sub>50</sub>) of < 1 ppm. However, they varied in EC<sub>90</sub> values. Twenty ppm concentrations of Amistar Top (Strobilurin+Azole) showed 90% effective concentration (EC<sub>90</sub>). Amistar (Strobilurin) also showed approximately same results as Amistar Top while EC<sub>90</sub> value of Natio, Score, Tilt, Lusture, Folicur, Contaf and Kavach was <25ppm. It was also observed Strobilurin+Azole group fungicides also dominant in the field condition to control the disease.

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