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# Survey of Anthracnose of Green Gram in East Nimar Region of Madhya Pradesh

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## Abstract

A roving survey to assess the incidence of Anthracnose of Greengram was undertaken. The overall mean incidence of disease was observed to be 26.06% (range–20.00% to 29.60%). indicating an alarming preparation of anthracnose in the vicinity of Khandwa (M.P.). Among the eight villages Lowest anthracnose incidence was noticed in Piplod (20.00 %) followed by Bir (24.16 %), while highest anthracnose incidence was recorded Jaswadi (29.60 %) followed by Attar (28.96 %). Variability in the cultural characters of different isolates of *C. truncatum* was studied by growing on potato dextrose agar medium. Different isolates varied in colony diameter, colony type and colony colour. Significant difference in the colony which varied from 60.33 mm to 79.67 mm. Highest mycelial growth was recorded in Isolate CC-1 (79.67 mm) while the least colony diameter was observed with the isolate CC-6 (60.33 mm). Similarly isolates categorized into two groups on the basis of colony texture viz., Flat and fluffy. Maximum of 5 isolates (CC-1, CC-2, CC-4, CC-6 and CC-7) produced fluffy colony while Flat colony texture was recorded in three isolates (CC-3, CC-5 and CC-8). Based on visual observation on colony colour, the cultures were divided into three groups. Dark grey colour colony was observed in 3 isolates (CC-1, CC-5 and CC-8), light grey (CC-2 and CC-7) and dull white (CC-3, CC-4 and CC-6). Morphologically isolates varied in size, colour, shape, production of their setae, acervuli and conidia. Mean of conidia length ranged from 21.47-25.22 ìm with maximum in CC-4 and minimum in CC-1. Significant variation was observed in the Width of conidia with maximum Width (4.32 m) in CC-4 and minimum (3.62 ìm) in CC-1.

***Key words :*** *Greengram, anthracnose, colletotrichum.*

## Introduction

Greengram *[Vigna radiata* (L.) Wilczek] is an important pulse crop of India. Apart from India it is widely cultivated throughout the South Asia including Bangladesh, Pakistan, Thailand, Sri Lanka, Cambodia, Vietnam, Malaysia, Indonesia, and South China. The major Greengram growing states of India are Maharashtra, Karnataka, Rajasthan, Orissa, Bihar, Madhya Pradesh, Uttar Pradesh, Tamil nadu, Punjab, West Bengal and Haryana. Greengram ranks third among all pulses grown in India after chickpea and pigeon pea (1). In India, Greengram is grown on 4.30 m ha and total production of 2.07 m tones of grains with productivity of 4.81 q/ha in 2016 (2). Madhya Pradesh produces more than seven per cent of total production of the country. Among various factors responsible for low yields, biotic and abiotic stresses take a heavy toll of the crop, out of which diseases cause an estimated yield loss of 20 to 30 per cent (3).

Greengram suffers from many diseases caused by fungi, bacteria, nematodes, viruses, and also abiotic stresses. Among the fungal diseases, powdery mildew, anthracnose, Cercospora leaf spot, web blight and dry root rot are most prevalent. In recent years, anthracnose caused by *Colletotrichum truncatum* (*Schw.*) Andrus and Moore has become one of the major diseases to occur in many countries viz. India, Nigeria, Thailand, Philippines, Upper volta, Zambia, Palmira, Columbia, etc. (4). It occurs in all the parts of the world, wherever greengram is cultivated. In India, the anthracnose of greengram was first reported from Jorhat Assam in 1951 (5). The disease has been reported from all major Greengram growing regions of India in mild to severe form and in tropical and subtropical areas it causes considerable damage by reducing seed quality and yield. The disease causes qualitative as well as quantitative losses (6). Losses in yield due to anthracnose have been estimated to be in the range of 24 to 67 per cent (6) and with the DI % 18.2 to 86.57 in northern Karnataka (8). The yield losses caused due to greengram anthracnose are proportional to the disease severity and vary remarkably with the stage of infection, genotypes and environmental conditions. The disease is characterized by serious leaf spotting ultimately resulting in 'shot hole' symptoms and finally defoliation which affects the yield greatly. Infection of pods directly damages the seeds and reduces its germination ability. Pod infection may result in complete loss in yield.

## Materials and Methods

**Survey :** A roving survey method was followed to assess the incidence of anthracnose of greengram in four major

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greengram growing blocks of East Nimar. The survey was conducted during Kharif 2018, minimum of two villages were selected from each blocks and in each village, minimum of two fields were selected to assess the incidence of greengram anthracnose. The per cent disease incidence of greengram anthracnose was computed.

**Isolation, purification and identification of pathogen :** The work of isolation and purification was carried out in laminar air flow, which was sterilized before going to use. The infected leaves and pods of greengram were cut into small bits, surface sterilized with 0.1% mercuric chloride solution ($HgCl_2$) followed by washing with distilled water two times and placing in BOD incubator for 2-3 days for fungus to grow after 2-3 days mycelial growth were seen, small pieces of mycelia were placed on already poured PDA (potato dextrose agar media) petriplates. The culture obtained was further purified by using hyphal tip method; cultures were maintained at 26±2°C in slants for further use. The pathogen was identified by using the standard manual (9).

**Cultural and morphological characteristics of pathogen :** Eight isolates of *Colletotrichum truncatum schw*. were obtained from major greengram growing blocks of East Nimar *viz.* Khandwa, Pandhana, Punasa and Chhegaon makhan that were aseptically grown separately on cooled PDA medium in sterile glass Petri plates (90 mm dia.) and incubated on BOD at 26 ± 2°C for two weeks (14 days). Observations on cultural characteristics (colony colour, growth pattern and colony characters) and conidial production were recorded after two weeks of incubation. The fungus *Colletotrichum truncatum* was grown on potato dextrose agar medium and morphological studies conducted. The morphological observations were taken by use of glass slides and lectophenol. The conidia acervuli and mycelium were stained with cotton blue.

## Results and Discussion

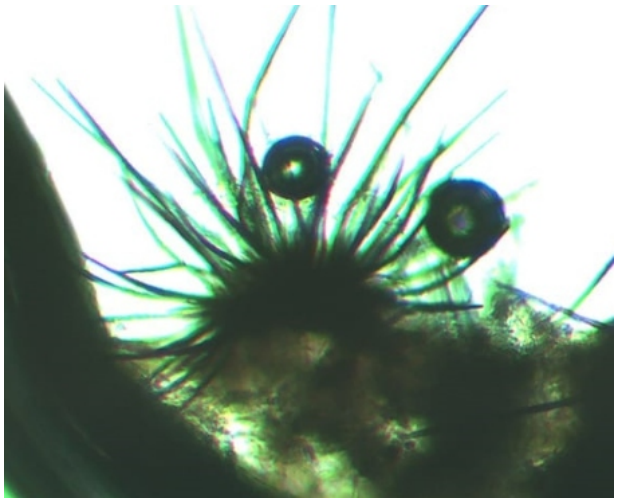
**Survey of disease incidence :** A survey was conducted in 2018 Kharif cropping season to assess the severity of anthracnose of greengram around the Khandwa district. During the survey, 08 places were visited and per cent disease incidence was recorded (Table-1). Total 08 places visited under 04 blocks of Khandwa districts *viz*., Khandwa, Pandhana, Punasa and Chhegaon makhan. During survey, anthracnose was found at all the sites and the incidence ranged from 20.00 to 29.60 per cent (Table-1).

Data presented in Table-1 revealed that Lowest anthracnose incidence was noticed in Piplod (20.00%) followed by Bir (24.16%), Singot (24.48%) while highest anthracnose incidence was recorded Jaswadi (29.60%) followed by Attar (28.96%), Dondwada (28.08%), Bangrada (27.20%) and Sirpur (26.00%). Among the blocks, minimum anthracnose incidence was recorded in Pandhana (22.24%) followed by Punasa (25.68%) and Khandwa (27.80%), while maximum anthracnose incidence was recorded in Chhegaon makhan (28.52%).

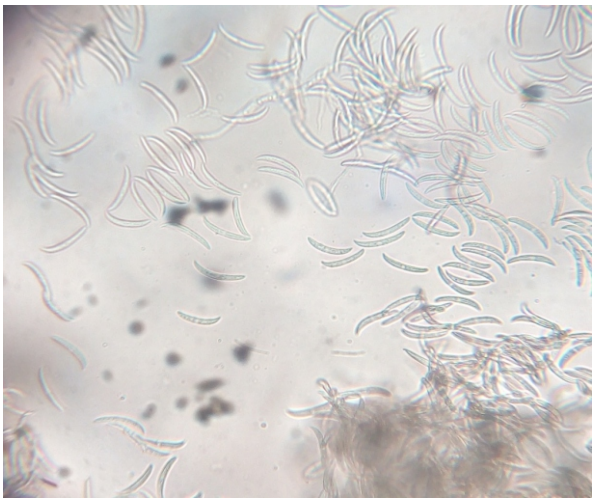
**Table-1 : Incidence of anthracnose of greengram at farmer's field in different blocks of Khandwa.**

| District | Block | Village | PDI (%) | Mean |
|---|---|---|---|---|
| Khandwa | Khandwa | Sirpur | 26.00 | 27.80 |
| | | Jaswadi | 29.60 | |
| | Pandhana | Singot | 24.48 | 22.24 |
| | | Piplod | 20.00 | |
| | Chhegaon makhan | Dondwada | 28.08 | 28.52 |
| | | Attar | 28.96 | |
| | Punasa | Bangrada | 27.2 | 25.68 |
| | | Bir | 24.16 | |
| | | | Mean | 26.06 |

**Isolation, purification, identification and maintenance of pure cultures :** Infected plant part of greengram leaves and pods collected from Khandwa district were used for the isolation of the fungus. The pathogenic fungus was isolated on potato dextrose agar medium and purified by single spore isolation method. After inoculation and incubation as described in the material and methods, the dull white to grey mycelial growth emerged from diseased leaf tissues on potato dextrose agar medium in Petri plates. The fungus got isolated by single spore technique and transferring them to fresh slants containing potato dextrose agar medium. Pure culture of the fungus was obtained by several such transfers and kept viable by sub-culturing at the interval of 21 days. The pure culture thus obtained was maintained in the refrigerator for further studies. The colonies of *Colletotrichum truncatum* were dark brown to black with septate, branched, brown and 3 to 4 m thick mycelium. The acervuli were black in colour, oval to conical in shape and measuring 171.5 X 248.0 ìm. The acervuli were embedded with in very light pinkish coloured mucilaginous mass containing numerous conidia. Setae were longer than conidiophore, erect, hairlike, broader at base and tapering at apex, black coloured and arising through the mucilaginous mass of conidia and measured 78.0 to 201.0 m X 5.0 – 7.1 ìm. Conidia were single celled, smooth walled, hyaline, curved and measured 20.0 to 23.3 m X 3.5 to 4.0 m in size, and germinate by germ tubes. The appressoria were sparse, pale to dark brown, clavate or circular in outline and 8 m X 6.7 m in size.



**Acervulus and Setae of the pathogen**



**Conidia of the pathogen**

**Symptomatology :** The spots first appeared on the lower surface of leaf, and later on the petioles, stem and pods. The spots were brown with reddish centers on lower surface. At later stage, spots appeared on upper surface with reddish brown ring of 8–10 mm diameter and subsequently turned. Dark brown chlorotic which later resulted in 'shot holes'. On lower surface, the symptoms developed as 10–12 mm diameter patches of bright blood red stains. Similarly, on petiole and stem, reddish brown colored streaks appeared. Identical symptoms appeared on pods yielding discolored and small seeds. In severe cases, the whole leaf was covered with brown patches. The spots coalesced to form large patches and in severe cases caused premature defoliation.

**Cultural and morphological characteristics :** Variability in the cultural characters of 8 isolates *of C. truncatum* was studied by growing on potato dextrose agar medium. Observations on various cultural characters were recorded as described in material and methods and the results obtained are presented in Table-2.

**Colony diameter :** The data on colony diameter presented in the Table-2 revealed that there was a significant difference in the colony which varied from 60.33 mm to 79.67 mm. Highest mycelial growth was recorded in Isolate CC-1 (79.67 mm) followed by CC-8 (78.00 mm), CC-7 (75.33 mm), CC-4 (72.00 mm) and CC-5 (71.33 mm), while the least colony diameter was observed with the isolate CC-6 (60.33 mm) followed by CC-2 (63.17 mm) and CC-3 (67.83 mm).

**Colony type :** Isolates categorized into two groups on the basis of colony texture *viz*., Flat and fluffy (Table-3). Maximum of 5 isolates (CC-1, CC-2, CC-4, CC-6 and CC-7) produced fluffy colony while Flat colony texture was recorded in three isolates (CC-3, CC-5 and CC-8).

**Colony colour :** Based on visual observation on colony colour, the cultures were divided into three groups. Dark grey colour colony was observed in 3 isolates (CC-1, CC-5 and CC-8), light grey (CC-2 and CC-7) and dull white (CC-3, CC-4 and CC-6) colour colony was observed in 3 isolates each respectively.

**Colony margins :** Isolates categorized into two groups on the basis of colony margins *viz.,* Smooth and Irregular (Table-3). Five isolates recorded Smooth colony margins (CC-1, CC-2, CC-3, CC-6 and CC-8), while three isolates recorded irregular colony margins (CC-4, CC-5, and CC-7).

Variability in the morphological characters of 8 isolates of C. truncatum was studied and observations on various morphological characters were recorded (Table-3).

**Length of conidia :** Data presented in Table-3 showed that the length of conidia varied from 21.47 ìm (CC-1) to 25.22 m (CC-4). Maximum length of conidia was observed in case of isolate CC-4 (25.22 m) followed by CC-6 (24.66 m), CC-8 (23.10 m), CC-3 (22.33 m) and CC-2 (22.07 m), while the isolate CC-1 observed minimum length of conidia (21.47 m) followed by CC-5 (21.70 m) and CC-7 (21.94 m).

**Width of conidia :** The width of conidia varied from 3.62 ìm (CC-1) to 4.32 m (CC-4) (Table-3). Maximum width of conidia was recorded in CC-4 (4.32 m) followed by CC-7 (4.22 m), CC-6 (4.00 m), CC-3 (3.98 m) and CC-2 (3.92 ìm), while minimum width of conidia was recorded in CC-1 (3.62 m) followed by CC-8 (3.84 m) and CC-5 (3.90 m).

A survey was conducted around Khandwa district and covered four blocks of Khandwa districts viz., Khandwa, Pandhana, Chhegaon makhan and Punasa. Anthracnose was found at all the sites and the incidence ranged from 20.00 to 29.60 per cent. Lowest anthracnose

**Table-2 : Variability in cultural characteristics of *Colletotrichum truncatum* isolates.**

| Place | Isolates code | Mycelial growth (mm)* | Colony type | Color of colony | Colony margins |
|---|---|---|---|---|---|
| Sirpur | CC-1 | 79.67 | Fluffy | Dark Grey | Smooth |
| Jaswadi | CC-2 | 63.17 | Fluffy | Light Grey | Smooth |
| Singot | CC-3 | 67.83 | Flat | Dull white | Smooth |
| Piplod | CC-4 | 72.00 | Fluffy | Dull White | Irregular |
| Dondwada | CC-5 | 71.33 | Flat | Dark Grey | Irregular |
| Attar | CC-6 | 60.33 | Fluffy | Dull white | Smooth |
| Bangrada | CC-7 | 75.33 | Fluffy | Light grey | Irregular |
| Bir | CC-8 | 78.00 | Flat | Dark grey | Smooth |
| SEm± | 0.59 | - | - | - | |
| C.D. at 5 % | 1.80 | - | - | - | |

**Table-3 : Variability in morphological characteristics of *Colletotrichum truncatum* isolates.**

| Place | Isolates code | Size of conidia (µm)* | | | |
|---|---|---|---|---|---|
| | | Length (µm) | | Width (µm) | |
| | | Mean | Range | Mean | Range |
| Sirpur | CC-1 | 21.47 | 20.20-22.10 | 3.62 | 3.10-4.10 |
| Jaswadi | CC-2 | 22.07 | 20.05-23.78 | 3.92 | 3.50-4.40 |
| Singot | CC-3 | 22.33 | 21.00-24.50 | 3.98 | 3.80-4.20 |
| Piplod | CC-4 | 25.22 | 24.60-25.80 | 4.32 | 4.00-4.50 |
| Dondwada | CC-5 | 21.70 | 20.10-23.55 | 3.90 | 3.40-4.40 |
| Attar | CC-6 | 24.66 | 23.62-25.40 | 4.00 | 3.70-4.40 |
| Bangrada | CC-7 | 21.94 | 20.00-23.44 | 4.22 | 3.80-4.50 |
| Bir | CC-8 | 23.10 | 21.50-24.61 | 3.84 | 3.50-4.00 |
| SEm± | | 0.50 | | 0.14 | |
| C.D. at 5% | | 1.45 | | 0.40 | |

*Mean of five replications

incidence was noticed in Piplod followed by Bir, Singot while highest anthracnose incidence was recorded Jaswadi followed by Attar, Dondwada, Bangrada and Sirpur. Among the blocks, minimum anthracnose incidence was recorded in Pandhana followed by Punasa and Khandwa, while maximum anthracnose incidence was recorded in Chhegaon makhan. Similar results were recorded by (8) he reported 18.2 to 86.57 per cent incidence of the mungbean anthracnose in North Karnataka. (10) lso conducted survey in major districts of northern Karnataka Province to assess the incidence of anthracnose of greengram and they observed 21.36 to 58.97 per cent disease severity under field conditions which was in accordance of present result.

Infected plant part of greengram leaves and pods collected from Khandwa district were used for the isolation of the fungus. The pathogenic fungus was isolated on potato dextrose agar medium and purified by single spore isolation method. After inoculation and incubation as described in the material and methods, the dull white to grey mycelial growth emerged from diseased leaf tissues on potato dextrose agar medium in Petri plates. The fungus got isolated by single spore technique and transferring them to fresh slants containing potato dextrose agar medium.

The colonies of *Colletotrichum truncatum* were dark brown to black with septate, branched, brown and 3 to 4 ìm thick mycelium. The acervuli were black in colour, oval to conical in shape and measuring 171.5 X 248.0 m. The acervuli were embedded with in very light pinkish coloured mucilaginous mass containing numerous conidia. Setae were longer than conidiophore, erect, hairlike, broader at base and tapering at apex, black coloured and arising through the mucilaginous mass of conidia and measured 78.0 to 201.0 ìm X 5.0 – 7.1 ìm. Conidia were single celled, smooth walled, hyaline, curved and measured 20.0 to 23.3 ìm X 3.5 to 4.0 ìm in size, and germinate by germ tubes. The appressoria were sparse, pale to dark brown, clavate or circular in outline and 8 ìm X 6.7 ìm in size. Similar results were recorded by (11) they were isolated *C. truncatum*, the incitant of anthracnose of mung bean on PDA and proved its pathogenicity. (12) also isolated *Colletotrichum truncatum*

and reported that PDA gave maximum mycelia growth of *C. truncatum* followed by Czapek-Dox-Agar.

Cultural variability among 8 isolates of *C. truncatum* was studied on the basis of the mycelial growth, colony type, color of colony and colony margins. The data on colony diameter showed there was a significant difference in the colony which varied from 60.33 mm to 79.67 mm. Highest mycelial growth was recorded in Isolate CC-1 (79.67 mm) followed by CC-8 (78.00 mm), CC-7 (75.33 mm), CC-4 (72.00 mm) and CC-5 (71.33 mm), while the least colony diameter was observed with the isolate CC-6 (60.33 mm) followed by CC-2 (63.17 mm) and CC-3 (67.83 mm). Based on visual observation on colony colour, the cultures were divided into three groups. Dark grey colour colony was observed in 3 isolates (CC-1, CC-5 and CC-8), light grey (CC-2 and CC-7) and dull white (CC-3, CC-4 and CC-6) colour colony was observed in 3 isolates each respectively. Similar results were recorded by (13) reported that among the eleven isolates collected from six different states exhibited cultural variability with respect to colony colour varied from cottony white to dark gray, spore ranging between 20.10-23.00 x 3.2-4.50 to 21.10-24.20 x 3.8-4.10 m. (14) also described that Colony colour was medium grey to dark grey, dense colony, reverse dark brown.

Morphological variability among eight isolates of C. truncatum was studied on the basis of the length of conidia, width of conidia, length of setae and width of setae. Length of conidia varied from 21.47 m (CC-1) to 25.22 m (CC-4). Maximum length of conidia was observed in case of isolate CC-4 (25.22 m) followed by CC-6 (24.66 m), CC-8 (23.10 m), CC-3 (22.33 m) and CC-2 (22.07 m), while the isolate CC-1 observed minimum length of conidia (21.47 m) followed by CC-5 (21.70 ìm) and CC-7 (21.94 m). The width of conidia varied from 3.62 m (CC-1) to 4.32 m (CC-4). Maximum width of conidia was recorded in CC-4 (4.32 m) followed by CC-7 (4.22 m), CC-6 (4.00 m), CC-3 (3.98 m) and CC-2 (3.92 m), while minimum width of conidia was recorded in CC-1 (3.62 m) followed by CC-8 (3.84 m) and CC-5 (3.90 m). (15) observed that the acervulie of the pathogen were black in colour, oval to conical in shape, measuring 171.5 × 248.0 µ setae were longer than conidiophore and measured 78.0-201.0 × 5.0-7.1 µ. Conidia were single celled, smooth walled, hyaline, curved and measured 20.0-23.3 × 3.5-4.0 µ in size. Similar results were recorded by (16). Conidial length and width among the test isolates varied from 18.81-26.46 m and 3.56-4.63 m respectively. Number of setae and there average length also varied from 48-158 and 44.36-166.65 m, respectively.

## Conclusions

Total eight places visited under four blocks of East Nimar region of Madhya Pradesh *viz.,* Khandwa, Pandhana, Chhegaon makhan and Punasa. During survey, anthracnose was found at all the sites and the incidence ranged from 20.00 to 29.60 per cent. Lowest anthracnose incidence was noticed in Piplod (20.00 %), while highest anthracnose incidence was recorded Jaswadi (29.60 %) Among the blocks, minimum anthracnose incidence was recorded in Pandhana, while maximum anthracnose incidence was recorded in chhegaon makhan. Highest mycelial growth was recorded in Isolate CC-1 followed by CC-8, while the least colony diameter was observed with the isolate CC-6. Maximum of 5 isolates (CC-1, CC-2, CC-4, CC-6, CC-7) produced fluffy colony while compact colony texture was recorded in three isolates (CC-3, CC-5, CC-8). Dark grey colour colony was observed in 3 isolates (CC-1, CC-8, CC-5), light grey (CC-2, CC-7) and dull white (CC-6, CC-4 CC-3. Maximum length of conidia was observed in case of isolate CC-4, while the isolate CC-1 observed minimum length of conidia. Maximum width of conidia was recorded in CC-4, while minimum width of conidia was recorded in CC-1.

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