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# Distribution of Charcoal Rot of Soybean, its Influencing Factors and Pathogenic Variabilities in Different Regions of Madhya Pradesh

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## ABSTRACT

**Background:** *Macrophomina phaseolina* (Tassi) Goid is a necrotrophic soil-inhabiting fungus that causes disease in several economic plants worldwide and has now become a serious threat to soybean cultivation in central India.

**Methods:** The random surveys were done during 2018 and 2019 to determine the present status of charcoal rot (*M. phaseolina*) in key soybean growing districts of Madhya Pradesh. Sixteen isolates of pathogen were characterized based on morpho-cultural and pathogenic variability by using cut stem inoculation techniques at J.N.K.V.V., Jabalpur.

**Result:** Charcoal rot was prevalent in all surveyed sixteen districts of Madhya Pradesh. Out of six, Kymore plateau and Satpura hills (44.5%), Satpura plateau (42.5%), Central Narmada Valley (42.0%) and Vindhyan plateau agroclimatic zones (35.0 %) were identified as favouable zones for charcoal rot. Malwa plateau (16.25%) and Northern hill region (24.5%) had low incidences of charcoal rot. Among varieties, JS 95-60, JS 93-05 and JS 20-29 were highly affected, whereas, JS 20-34, JS 20-98 and JS 20-69 were least affected. Incidence of charcoal rot was comparatively higher in the fields that had Maize-Chickpea (26.32 %), Maize-Wheat, Soybean-Pea and Soybean - Chickpea cropping patterns in the previous year and lowest in Rice-Chickpea (12.00%) and Rice - Wheat (12.45%) cropping patterns. The incidence of charcoal rot was partially higher in the fields with light soil (21.3%) than in heavy soil (19.0%). Isolates investigation revealed that isolates from Jabalpur, Hoshangabad, Chhindwara and Sagar were fast-growing variables and highly aggressive in developing necrotic lesions on the cut stem of soybean. This investigation could be instrumental in forewarning sensitive areas of charcoal rot, varietal options and crop rotation to be followed to minimize the incidence of charcoal rot. Moreover, identified variable aggressive isolates could be utilized in genotype resistance screening programs.


**Key words:** Charcoal rot, Cropping pattern, Incidence, *Macrophomina phaseolina,* Soybean, Variability.

## INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is a seed legume cultivated as a leading oilseed crop worldwide. It is one of the richest vegetarian sources of proteins (36.1-42.8%) and edible oil (16.8-20.2%), along with several beneficial contents, including amino acids, unsaturated fatty acids, minerals (Ca and P), vitamins (*e.g.* A, B, C and D), antioxidants which make it one of the best food and feed for human and other animals (Kumar *et al*., 2020; Mehra *et al*., 2020; Banerjee *et al*., 2023). India is among the top fifth producers of soybean in the world. Nevertheless, it is simultaneously the largest soybean oil importer, accounting for about $5.6B (OEC, 2023). The central part of the country that includes Madhya Pradesh (45%), Maharashtra (38%) and Rajasthan (8%) are major soybean growing regions. The soybean productivity in India is far lower (about 0.9 tons) compared to other countries like USA, Brazil and Argentina (3.0 to 3.5 tons) (SOPA, 2023). In India, soybean is a *Kharif* season crop cultivated in rainfed conditions during which the crop succumbed to various abiotic and biotic stresses (Dupare *et al*., 2014; Amrate *et al*. 2021; Amrate *et al*. 2023a). In this concern, sustainable soybean production in the central part of the country has suffered due to the attack of many diseases such as yellow mosaic, charcoal rot, aerial blight, anthracnose, bacterial pustule, frog eye leaf spot *etc* (Amrate *et al*., 2020a; Amrate and Shrivastava, 2021; Rajput *et al*., 2021; Uikey *et al*. 2022; Amrate *et al*., 2023b).

In this, charcoal rot caused by a necrotrophic soil-borne fungus *Macrophomina phaseolina* (Tassi) Goid is a devastating disease of soybean responsible for significant

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yield reduction in the top eight soybean-producing nations (Wrather *et al*., 2010; Amrate *et al*., 2023c). It occurs yearly and affects most genotypes, varieties and advanced soybean lines under Madhya Pradesh agro conditions (Amrate *et al*., 2019; Amrate and Shrivastava, 2021; Amrate *et al*., 2023c). Sudden wilting of plants and greyish-black appearance of lower stems and roots are common symptoms of charcoal rot (Gupta *et al*., 2012; Amrate *et al*., 2020b; Sagarika *et al*., 2023). *Macrophomina phaseolina* has a wide host range (about 500), including field crops, vegetables, fruit, ornamental, medicinal and forest plants (Marquez *et al*., 2021; Amrate *et al*., 2023c). Charcoal rot is challenging to manage due to its complex pathogenesis and sudden onset of wilting symptoms (Sagarika *et al*., 2023; Singh *et al*., 2024). This particular pathogen can cause significant economic losses in crop plants. Recent reports from several parts of Madhya Pradesh indicated that soybean crops had been facing the problem of sudden wilting during reproductive stages and, thereby, significant yield loss in those fields. However, more information was needed on the extent of severity and distribution of charcoal rot and pathogens behaviour in the critical soybean-producing state of the country. Because of this, a comprehensive survey was conducted to record the prevalence and distribution of charcoal rot and possible factors associated with it in the state. Furthermore, the cultural and pathogenic nature of isolates belonging to surveyed regions was also determined. The study could be helpful for farmers and researchers in providing better varietal options and other preventive measures for minimizing charcoal rot incidence. The most pathogenic isolate could also be used in an artificial resistance screening programme for charcoal rot.

## MATERIALS AND METHODS

### Survey locations and methodology

A comprehensive disease survey was conducted to record the incidence and distribution of soybean charcoal rot at farmers' fields in twenty-two blocks of sixteen districts representing six agro-climatic zones of Madhya Pradesh during *kharif* 2018 and 2019. A random survey was conducted at the reproductive stages of soybean (R5-R7), coinciding with the second fortnight of September. Roadside, easily approachable farmer's fields were selected and 400 plants were observed at four randomly selected points from each field. Charcoal rot-affected plants were identified based on the typical symptoms of charcoal rot (Amrate *et al*., 2020b) (Fig 1) and the association of the pathogen was confirmed in the lab (Fig 2). The information on soybean varieties, soil types and the previous year's cropping pattern of the same piece of land was also obtained by interacting with farmers. The diseased samples were collected and brought to the lab for further studies. After the survey, blocks, districts and variety wise per disease incidence of charcoal rot were calculated by using the formula given below:

$$\text{Per cent incidence} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

To interpret the influence of cropping patterns on charcoal rot, fields with similar cropping patterns during the previous year were averaged for two years for charcoal rot incidence across the zones. Similarly, the fields were grouped mainly as heavy soil (dark to medium black) and light soil (Sandy and sandy loam soil) and the average incidence of charcoal rot on those fields was also computed.

### Cultural and morphological variability among different isolates

The pathogenic fungus was isolated, purified and identified using standard techniques on potato dextrose medium (Marquez *et al*., 2021) (Fig 2). The isolates from districts were assigned a unique code name. The radial growth of isolates on PDA was measured four days after inoculation at 26±1°C. Isolates were classified as fast (>85 mm), medium (75-85 mm) and slow (<75 mm) based on the growth, respectively. The growth pattern and colour of the colony were recorded on the 7th and 10th day after inoculation, respectively. Five mm five mycelium mats of ten days old culture from all corners, including the centre, were suspended in 5 ml of sterile distilled water. After that, one drop of the suspension was examined under a stereo binocular microscope for microslcerotial shape and their numbers. The number of sclerotia per microscopic (10X) field was counted from five microscopic fields for three replications (three culture plates) for each isolate.

### Testing of pathogenic variability by using cut stem inoculation technique

Eighteen days old seedlings of JS 20-29 were inoculated by the actively growing culture of isolates of *M. phaseolina* by using the cut stem inoculation technique (Twizeyimana *et al*., 2012; Amrate *et al*., 2019). Initially, plants were cut at 3.0 cm above the unifoliolate node and cut end, then inoculated by three days actively growing culture on PDA (Fig 3). For inoculation, the open end of the sterile micropipette tip (10 to 200 µl) was inserted into the medium. A circular disc of fungal mycelium was cut, picked up and immediately placed over the cut stem. After the 3$^{rd}$, 6$^{th}$, 9$^{th}$ and 12$^{th}$ days of inoculation, downward linear stem necrosis was measured. Four plants of each variety were inoculated for this purpose. The experiment was conducted under polyhouse conditions (temperature 30°C±3 and RH >75%).

### Calculation of Area under lesion progression curve (AULPC)

By using linear stem necrosis length at 3$^{rd}$, 6$^{th}$, 9$^{th}$ and 12$^{th}$ days, area under lesion progression curve (AULPC) was calculated as given by Shaner and Finney (1977).

$$AULPC = {}^{n-1}\Sigma_{i=1} [(y_i + y_{i+1})/2][t_{i+1}-t_i]$$

Where,

$y_i$= Necrotic lesion at i$^{th}$ observation.

$t_i$= Time (days) at i$^{th}$ observation.

n= Number of observations.

## RESULTS AND DISCUSSION

### Incidence of charcoal rot across the zones

The charcoal rot was found in all the major soybean growing areas of Madhya Pradesh (Table 1). In Kymore Plateau and Satpura hills agroclimatic zones, the incidence of charcoal rot ranged between 1.75-44.50 (in 2018) and 10.75-34.25% (in 2019), with the maximum average of 39.38% in JS 95-60 at Seoni block, Seoni. In Satpura plateau agroclimatic zone, it was distributed from 1.25-42.50 and 0.50-32.00% during 2018 and 2019, respectively. Variety JS 20-29 (34.50%) was highly affected at Mohkhed, Chhindwara. In Central Narmada Valley, it ranged between 0.75-42.0 and 0.50-32.25% during 2018 and 2019, respectively, with the highest average (37.13%) in JS 95-60 at Hoshangabad. In the Vindhyan plateau, charcoal rot incidence varied from 2.25-35.0 and 0.75-31.0% during 2018 and 2019, respectively, with the maximum average (30.75%) in JS 95-60 at Vidisha. In the case of the Malwa plateau, charcoal rot was very low and ranged from 4.5-10.5 and 1.5-16.25% during 2018 and 2019, respectively. The JS 20-98 fields were free from charcoal rot infection. In the Northern hill region, it was also comparatively low and ranged between 13.0-24.5 and 1.75-19.25% in 2018 and 2019, respectively.

**Fig 1:** A field of view of charcoal rot affected plants.

Zone-wise average incidence was higher in Satpura Plateau (19.07%), followed by Kymore Plateau and Satpura Hills (18.81%) and central Narmada Valley (15.60%). At the same time, it was lowest in the Malwa Plateau (5.94%), *i.e.* Rajgarh and Ujjain district. Incidence was higher in 2018 (16.48 %) than in 2019 (12.26%). Across the zones, JS 95-60 was found to be the most affected (27.76 %), followed JS 93-05 (25.38%) and JS 20-29 (23.96%). At the same time, JS 20-34 (0.77%), JS 20-98 (0.83%) and JS 20-69 (1.70%) were least affected by charcoal rot. Our results have been corroborated with the findings of some recent investigations. Mishra and Dantre (2017) indicated that charcoal rot (30-60%) have also been infecting soybean

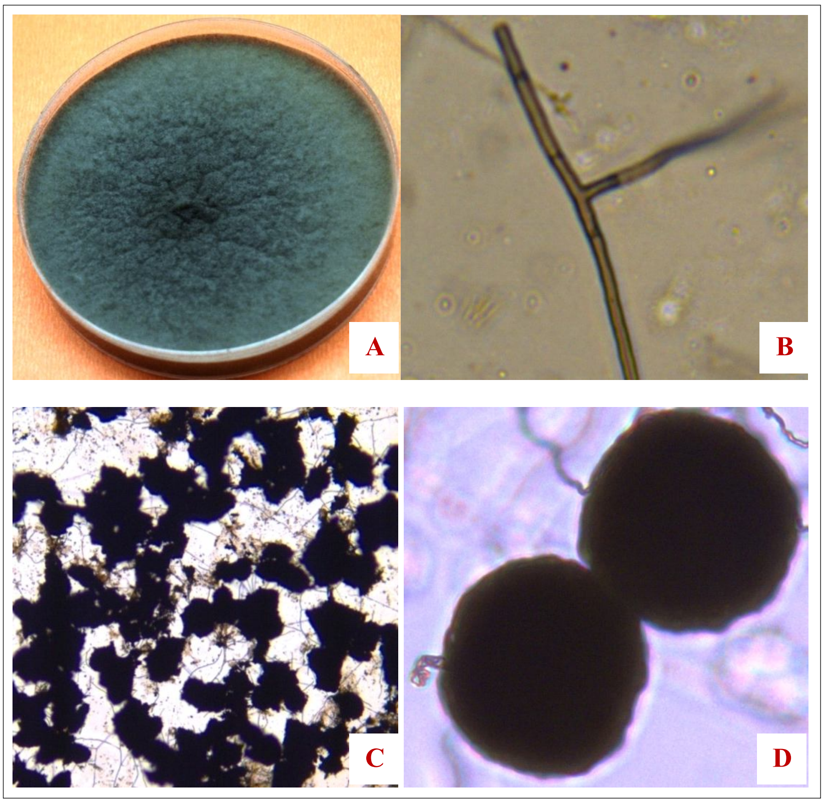


**Fig 2:** Cultural characteristic of *M. phaseolina*: ten days old culture (A), right angle branching of hyphae (B), presence of abundance microsclerotia (C) and close up view of round shaped microsclerotia (D), respectively.

varieties to varying extent in different districts of Chhattisgarh. High severity of charcoal rot (up to 100 %) in most presently growing varieties and germplasm at the experimental area in Jabalpur has also indicated its prevalence in the state (Amrate *et al*., 2018; Amrate *et al*., 2019). Charcoal rot is severe under high temperatures and erratic rainfall during reproductive stages in Indian conditions (Wrather *et al*., 2010; Gupta *et al*., 2012). The high incidence of charcoal in 2018 might be attributed to low rainfall and dry spells in most of the region of Madhya Pradesh, whereas in 2019, the conditions were not favourable.

**Influence of cropping pattern and soil type**

Through cropping pattern revealed that the incidence of charcoal rot was highest in the fields that had maize-chickpea (26.32%) cropping pattern in the previous year, followed by Maize-Wheat (23.75 %), Soybean - Pea (23.63%) and Soybean - Chickpea (18.87%) in the same land (Fig 4). The lowest average incidence of charcoal rot was recorded in the cropping pattern of rice-chickpea (12.00%) and Rice - Wheat (12.45%). The charcoal rot incidence was comparatively higher in the field with single/both crops (*kharif* and *rabi*) as hosts of charcoal rot pathogen. Maize and chickpea are the prominent host of *M. phaseolina*, which causes considerable losses in Indian conditions (Kaur *et al*., 2022; Bankoliya *et al*., 2022). Hence, it might be possible that these cropping patterns enhanced the pathogen load in soil, resulting in high disease. In contrast, a low incidence of charcoal rot in the Rice - Wheat system might reduce the microsclerotial of *M. phaseolina,* which could result in low infection in other hosts. Previous reports also indicate that crop rotation with non-host crops or less susceptible varieties have been influential in reducing inoculums of *M. phaseolina* (Gupta *et al*., 2012). Besides this, charcoal rot was partially higher in the field with light soil (6.5 - 21.3%) than in heavy soil (6.6 - 19.0%) across the agroclimatic zones and years (Fig 5). However, some fields with heavy soil also had a higher incidence than light soil in the same zone. Therefore, it could not be concluded that soil types influenced the incidence of charcoal rot disease in soybean.

**Cultural and morphological characteristics of isolates**

Mycelial growth among all isolates (16) was significantly varied from 76.33 (MP-13$_{(SHD)}$) to 90.00 (MP-9$_{(SGR)}$) mm (Table 2). Seven isolates *i.e.* MP-1$_{(JBP)}$, MP-2$_{(NAR)}$, MP-4$_{(CWA)}$, MP-6$_{(HBD)}$, MP-3$_{(SEO)}$, MP-9$_{(SGR)}$ and MP-14$_{(SHR)}$ were designated as Fast-Growing Isolates (FGI). Colony elevation patterns of most of the isolates were partially fluffy to fluffy (Fig 6). The colony

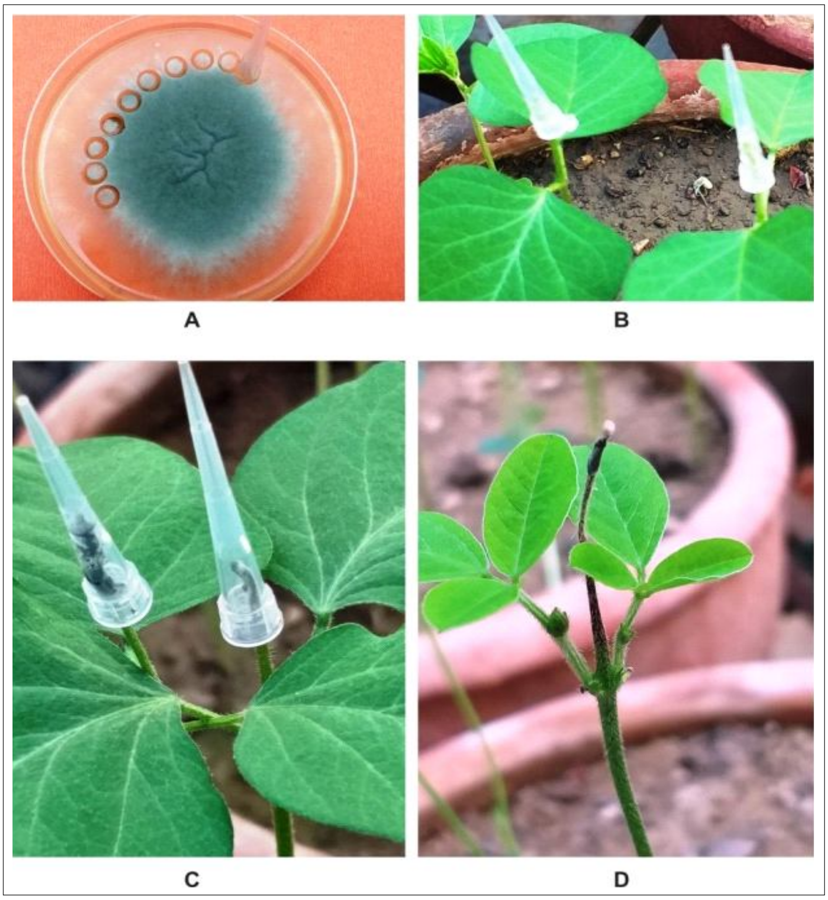


**Fig 3:** Cut stem inoculation technique**:** picking actively growing *M. phaseolina* culture (A), placing at cut end of stem (B), necrotic lesion in 2 days after inoculation (C) and Progression of lesion continued (D), respectively.

**Table 1:** Incidence of charcoal rot of soybean at different locations covering sixteen districts of six Agroclimatic zones of Madhya Pradesh during 2018 and 2019.

| *ACZ | Districts | Blocks | Variety | Charcoal rot (%) | | |
|---|---|---|---|---|---|---|
| | | | | 2018 | 2019 | Average |
| 1 | Jabalpur | Shahpura | JS 20-34 | 0.00 | 0.00 | 0.00 |
| | | | RVS 2001-04 | 18.50 | 10.75 | 14.63 |
| | | | JS 20-69 | 4.25 | 0.00 | 2.13 |
| | | | JS 93-05 | 26.75 | 17.15 | 21.95 |
| | | Patan | JS 20-34 | 0.00 | 0.00 | 0.00 |
| | | | JS 95-60 | 30.50 | 18.00 | 24.25 |
| | | | JS 20-29 | 39.50 | 23.00 | 31.25 |
| | Seoni | Seoni | JS 97-52 | 38.00 | 15.25 | 26.63 |
| | | | JS 20-29 | 42.50 | 24.00 | 33.25 |
| | | | JS 95-60 | 44.50 | 34.25 | 39.38 |
| | | | JS 93-05 | 35.00 | 27.75 | 31.38 |
| | | | JS 20-34 | 1.75 | 0.00 | 0.88 |
| | Zone wise average incidence | | | 23.44 | 14.18 | 18.81 |
| | Zone wise range of incidence | | | 0.0 0-44.50 | 0.0-34.25 | 0.0-39.38 |
| 2 | Chhindwara | Chaurai | JS 93-05 | 30.50 | 32.00 | 31.25 |
| | | | Js 20-34 | 2.25 | 0.50 | 1.38 |
| | | | JS 20-29 | 40.50 | 28.50 | 34.50 |
| | | Mohkhed | JS 20-29 | 38.00 | 31.00 | 34.50 |
| | | | JS 95-60 | 42.50 | 25.00 | 33.75 |
| | | | RVS 2001-04 | 22.00 | 16.75 | 19.38 |
| | Betul | Betul | JS 20-29 | 31.50 | 17.00 | 24.25 |
| | | | JS 93-05 | 36.75 | 23.50 | 30.13 |
| | | | JS 20-98 | 1.25 | 0.00 | 0.63 |
| | | | JS 20-34 | 2.75 | 1.50 | 2.13 |
| | | | JS 20-69 | 3.00 | 1.25 | 2.13 |
| | | | MACS 1188 | 26.00 | 17.50 | 21.75 |
| | | | RVS 2001-18 | 11.25 | 3.50 | 7.38 |
| | | Multai | JS 20-29 | 27.00 | 30.50 | 28.75 |
| | | | NRC 86 | 3.00 | 6.00 | 4.50 |
| | | | JS 93-05 | 25.75 | 31.50 | 28.63 |
| | Zone wise average incidence | | | 21.50 | 16.63 | 19.07 |
| | Zone wise range of incidence | | | 1.25-42.50 | 0.00-32.00 | 0.63-34.50 |
| 3 | Narsinghpur | Gotegoan | JS 20-34 | 0.00 | 0.00 | 0.00 |
| | | | JS 20-69 | 5.00 | 0.00 | 2.50 |
| | | | JS 20-29 | 25.50 | 17.25 | 21.38 |
| | | | RVS 2001-4 | 17.50 | 5.00 | 11.25 |
| | | | NRC 86 | 4.50 | 7.75 | 6.13 |
| | | | Pratap Soya 45 | 17.00 | 24.00 | 20.50 |
| | | Narsinghpur | NRC 86 | 3.00 | 2.25 | 2.63 |
| | | | JS 20-98 | 3.75 | 0.00 | 1.88 |
| | | | JS 20-29 | 33.50 | 20.00 | 26.75 |
| | | | RVS 2001-18 | 16.00 | 9.50 | 12.75 |
| | Hoshangabad | Hoshangabad | JS 20-34 | 0.75 | 0.00 | 0.38 |
| | | | JS 20-69 | 1.50 | 1.00 | 1.25 |
| | | | JS 20-29 | 36.50 | 26.00 | 31.25 |
| | | | JS 95-60 | 42.00 | 32.25 | 37.13 |
| | | Seoni Malwa | JS 95-60 | 34.00 | 29.00 | 31.50 |
| | | | RVS 2001-4 | 11.50 | 6.00 | 8.75 |

**Table 1: Continue....**

**Table 1: Continue....**

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | JS 93-05 | 33.25 | 25.00 | 29.13 |
| | Harda | Timarni | JS 95-60 | 41.00 | 31.75 | 36.38 |
| | | | JS 20-29 | 27.00 | 23.00 | 25.00 |
| | | | RVS 2001-4 | 11.00 | 8.25 | 9.63 |
| | | | JS 93-05 | 28.75 | 12.50 | 20.63 |
| | Sehore | Budni | JS 20-34 | 2.50 | 0.50 | 1.50 |
| | | | JS 95-60 | 31.50 | 27.25 | 29.38 |
| | | | RVS 2001-18 | 10.00 | 3.50 | 6.75 |
| | Zone wise average incidence | | | 18.21 | 12.99 | 15.60 |
| | Zone wise range of incidence | | | 0.00-42.00 | 0.00-32.25 | 0.38-37.13 |
| 4 | Sagar | Garhakota | JS 20-29 | 21.50 | 19.00 | 20.25 |
| | | | JS 20-69 | 0.00 | 2.00 | 1.00 |
| | | | JS 93-05 | 23.25 | 20.50 | 21.88 |
| | | Sagar | JS 20-34 | 0.00 | 0.75 | 0.38 |
| | | | JS 20-29 | 24.75 | 18.00 | 21.38 |
| | | | JS 95-60 | 31.50 | 17.00 | 24.25 |
| | | | JS 20-69 | 4.50 | 0.00 | 2.25 |
| | Damoh | Damoh | JS 93-05 | 24.50 | 17.00 | 20.75 |
| | | | JS 20-29 | 16.75 | 11.50 | 14.13 |
| | | | JS 20-34 | 0.00 | 0.00 | 0.00 |
| | | | JS 20-69 | 2.25 | 0.00 | 1.13 |
| | | | JS 97-52 | 26.25 | 14.00 | 20.13 |
| | Vidisha | Vidisha | JS 95-60 | 35.00 | 26.50 | 30.75 |
| | | | JS 20-29 | 20.50 | 16.00 | 18.25 |
| | | | JS 20-34 | 2.50 | 1.00 | 1.75 |
| | Raisen | Obedullaganj | JS 20-29 | 6.00 | 8.50 | 7.25 |
| | | | JS 95-60 | 23.25 | 31.00 | 27.13 |
| | | | JS 20-69 | 0.00 | 2.50 | 1.25 |
| | | | RVS 2001-04 | 8.00 | 11.25 | 9.63 |
| | Zone wise average incidence | | | 14.24 | 11.39 | 12.82 |
| | Zone wise range of incidence | | | 0.00-35.00 | 0.00-31.00 | 0.00-30.75 |
| 5 | Rajgarh | Narsinghgarh | RVS 2001-18 | 6.00 | 2.00 | 4.00 |
| | | | JS 95-60 | 10.50 | 16.25 | 13.38 |
| | | | JS 20-98 | 0.00 | 0.00 | 0.00 |
| | | | NRC 86 | 5.50 | 11.30 | 8.40 |
| | Ujjain | Ujjain | JS 20-34 | 0.00 | 1.50 | 0.75 |
| | | | RVS 2001-04 | 4.50 | 2.00 | 3.25 |
| | | | RVS 2001-18 | 7.75 | 4.25 | 6.00 |
| | | | JS 95-60 | 8.50 | 15.00 | 11.75 |
| | Zone wise average incidence | | | 5.34 | 6.54 | 5.94 |
| | Zone wise range of incidence | | 0.00-10.50 | 0.00-16.25 | 0.00-13.38 | |
| 6 | Umaria | Karkeli | JS 93-05 | 22.00 | 14.25 | 18.13 |
| | | | JS 335 | 21.50 | 10.25 | 15.88 |
| | | | JS 20-29 | 13.00 | 9.50 | 11.25 |
| | Shahdol | Sohagpur | JS 335 | 15.75 | 16.00 | 15.88 |
| | | | JS 95-60 | 24.50 | 19.25 | 21.88 |
| | | | JS 20-34 | 0.00 | 1.75 | 0.88 |
| | Zone wise average incidence | | | 16.13 | 11.83 | 13.98 |
| | Zone wise range of incidence | | | 0.00-24.50 | 1.75-19.25 | 0.88-21.88 |

*ACZ= Agroclimatic zone, 1= Kymore Plateau and Satpura Hills agroclimatic zone, 2= Satpura Plateau agroclimatic zone, 3= Central Narmada Valley agroclimatic zone, 4= Vindhyan Plateau agroclimatic zone, 5= Malwa Plateau agroclimatic zone and 6= Northern Hill Region zone.

colour of the isolates varied from dark black or black [MP-1$_{(JBP)}$, MP-3$_{(SEO)}$, MP-4$_{(CWA)}$, Mp-11$_{(VDS)}$ and Mp-13$_{(SHD)}$] to greyish [Mp-5$_{(BET)}$ and Mp-10$_{(RSN)}$] (Fig 6). Most of the isolates had oblong shape microsclerotia, except some had round. The number of sclerotia /microscopic fields (10x) varied significantly in most of the isolates and ranged from 41.07 [Mp-10$_{(RSN)}$] to 77.20 [Mp-4$_{(CWA)}$]. Four isolates, *i.e.* Mp-4$_{(CWA)}$, Mp-1$_{(JBP)}$, Mp-2$_{(NAR)}$ and Mp-14$_{(SHR)}$, had more than 70.0 sclerotia per microscopic field. Our findings revealed that isolates were variable concerning growth pattern, colony appearance and morphological traits, which are in agreement with the previous finding that also depicted variabilities in *M. phaseolina* isolated from different areas (Aghakhani and Dubey, 2009; Iqbal and Mukhtar, 2014; Gade *et al.*, 2018).

### Pathogenic variability

Progression of downward necrotic lesion through cut stem inoculation of all isolates differed at 3, 6, 9 and 12 days after inoculation in the variety JS 20-29. The Jabalpur isolates Mp-1$_{(JBP)}$ was found to be highly aggressive as recorded high length necrotic lesion of 15.30 cm, followed by Hoshangabad Mp-6$_{(HBD)}$ and Sagar Mp-9$_{(SGR)}$ isolates (12.80 cm) at 12 days after inoculation (Table 2). The isolates from Damoh Mp-8$_{(DMH)}$, Raisen Mp-10$_{(RSN)}$ and Umaria Mp-12$_{(UMR)}$ were the least aggressive, with the most downward,

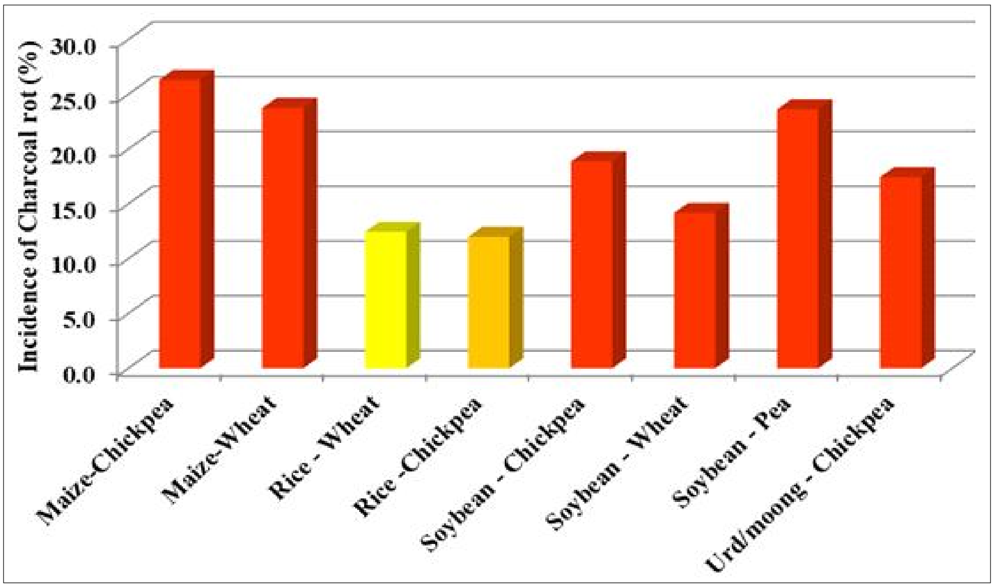


**Fig 4:** Influence of previous year cropping pattern on incidence of charcoal rot in soybean across the zones and years (2018 and 2019).

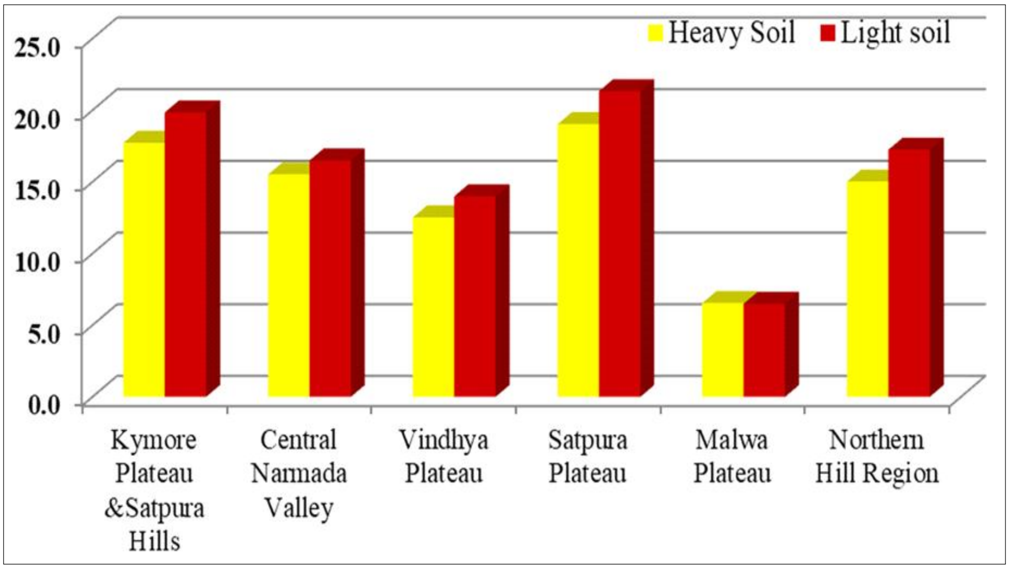


**Fig 5:** Influence of soil type on incidence of charcoal rot across the years (2018 and 2019).

**Table 2:** Cultural, morphological and pathogenic variability in the isolates of *M. phaseolina*.

| Location of isolates | | | | Cultural character | | | Sclerotia character (10 days) | | Pathogenic variability by CSI | |
|---|---|---|---|---|---|---|---|---|---|---|
| Places | Latitude | Longitude | Isolates | Mycelia growth (mm) | Colony texture | Colony color | Shape | Microsclerotia /microspic field (10×) | Lesion size | AULPC |
| J.N.K.V.V., Jabalpur(E) | 23°12′42″N | 79°56′53″E | Mp-1$_{(JBP)}$ | 89.00*** | PF | Dark black | Oblong | 76.83 | 15.30 | 80.55 |
| Gotegon, Narsingpur(F) | 23°02′15″N | 79°29′59″E | Mp-2$_{(NAR)}$ | 88.33*** | PF | Greyish black | Oblong | 71.93 | 9.50 | 57.45 |
| K.V.K., Seoni(E) | 22°04′05″N | 79°32′37″E | Mp-3$_{(SEO)}$ | 86.00*** | PFS | Dark black | Oblong | 69.67 | 9.00 | 52.20 |
| Mohkhede, Chhindwara(F) | 21°55′00″N | 78°56′13″E | Mp-4$_{(CWA)}$ | 88.33*** | fluffy | Dark black | Oblong | 77.20 | 11.40 | 63.75 |
| Betul Bazar, Betul(F) | 21°54′10″N | 77°55′16″E | Mp-5$_{(BET)}$ | 82.67** | PF | Greyish | Round | 46.00 | 10.15 | 60.08 |
| SeoniMalwa, Hoshangabad(F) | 22°29′24″N | 77°31′11″E | Mp-6$_{(HBD)}$ | 86.00*** | Fluffy | Greyish black | Oblong | 53.47 | 12.80 | 75.60 |
| Timarni, Harda(F) | 22°22′52″N | 77°14′59″E | Mp-7$_{(HRD)}$ | 84.00** | PF | Greyish black | Round | 45.30 | 12.40 | 72.00 |
| Damoh block, Damoh(F) | 23°48′57″N | 79°23′50″E | Mp-8$_{(DMH)}$ | 82.33** | Flat | Greyish black | Oblong | 43.43 | 3.00 | 24.90 |
| Z.A.R.S., Sagar(E) | 23°50′07″N | 78°41′54″E | Mp-9$_{(SGR)}$ | 90.00*** | Flat | Greyish black | Oblong | 66.67 | 12.80 | 70.65 |
| Obedullaganj, Raisen(F) | 22°59′18″N | 77°35′35″E | Mp-10$_{(RSN)}$ | 78.33** | PF | Greyish | Oblong | 41.07 | 2.80 | 21.75 |
| Vidisha block, Vidisha(F) | 23°30′37″N | 77°45′57″E | Mp-11$_{(VDS)}$ | 83.67** | PF | Black | Oblong | 57.60 | 8.50 | 48.60 |
| Karkeli, Umaria(F) | 23°27′46″N | 80°54′45″E | Mp-12$_{(UMR)}$ | 78.67** | Flat | Greyish black | Oblong | 48.07 | 2.95 | 22.95 |
| Sohagpur, Shahdol(F) | 23°19′23″N | 81°23′22″E | Mp-13$_{(SHD)}$ | 76.33** | Flat | Black | Oblong | 56.67 | 5.25 | 32.78 |
| Budni, Sehore(F) | 22°46′18″N | 77°39′58″E | Mp-14$_{(SHR)}$ | 86.00*** | Fluffy | Greyish black | Oblong | 73.27 | 6.00 | 37.28 |
| Narsinghgarh, Rajgarh(F) | 23°41′55″N | 77°04′15″E | Mp-15$_{(RJG)}$ | 83.33** | Flat | Greyish black | Round | 58.40 | 9.50 | 56.10 |
| Ujjain block, Ujjain (F) | 23°04′20″N | 75°49′20″E | Mp-16$_{(UJN)}$ | 84.00** | Flat | Greyish black | Round | 65.50 | 9.70 | 58.65 |
| - | - | - | CD (p=0.05) | 2.046 | - | - | - | 6.774 | - | - |
| - | - | - | SEM | 0.707 | - | - | - | 2.341 | - | - |

***Fast (>85 mm), **Medium (75-85 mm) and *slow (<75 mm) growing culture, PF= Partially fluffy, PFS= Partially fluffy scattered, CSI= Cut stem inoculation, *AULPC derived through lesion progression at 3, 6, 9 and 12 days after inoculation, Superscript letters F and E indicates farmer and experimental field, respectively.

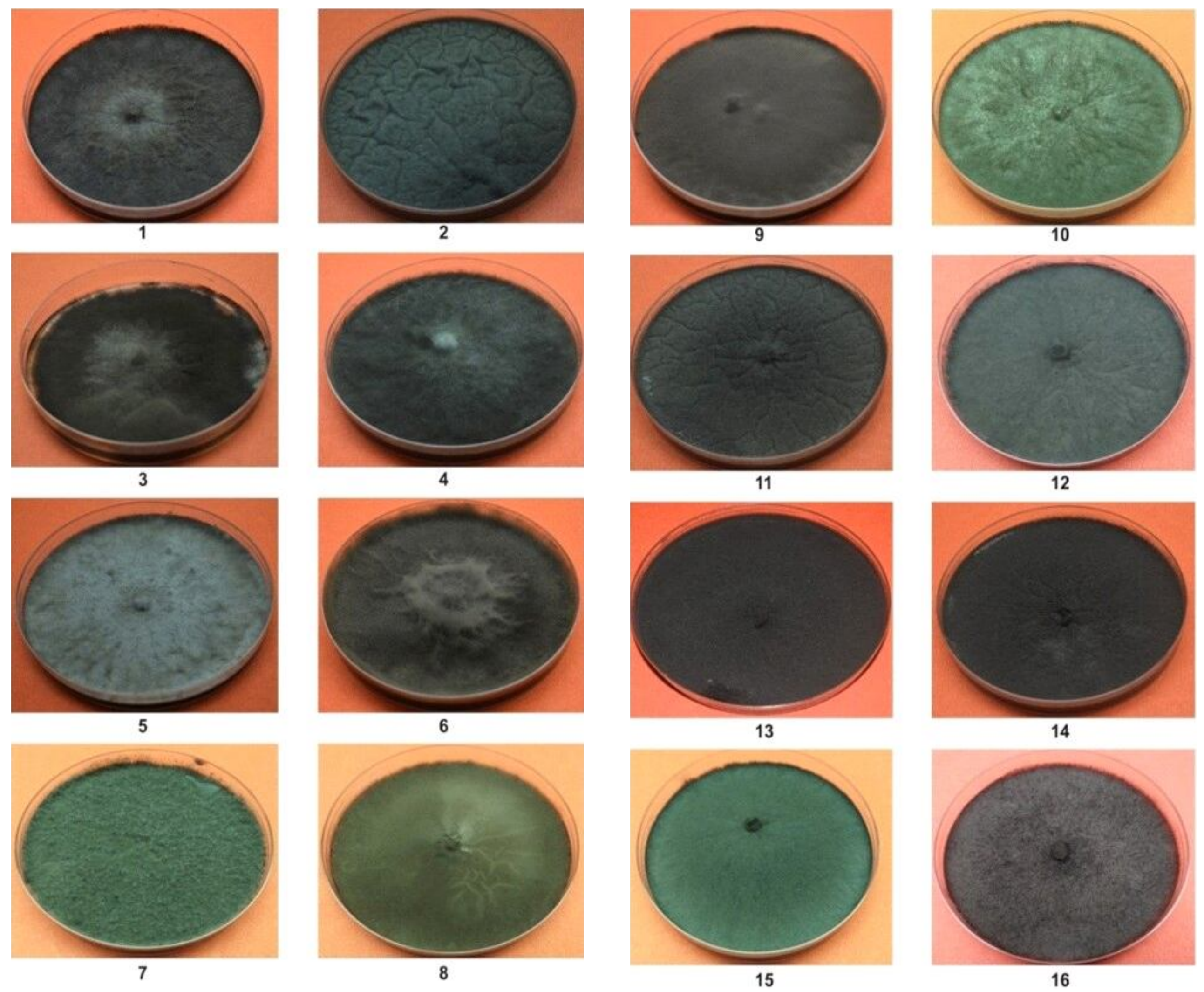


**Fig 6:** Variation in colony color and pattern of elevation of mycelium among *M. phaseolina* isolates (Mp - 1 to Mp - 16).

about 3.00 mm lesion. Similar trends were also depicted in the area under the lesion progression curve (AULPC). Through this, it was concluded that isolates from Jabalpur and Hoshangabad were the most aggressive among all sixteen. Previous to this, significant difference in necrotic lesion development and relative area under the disease progress curve by isolates of *M. phaseolina* from soybean was well described by Twizeyimana *et al.* (2012). Varieties also differed in necrotic lesion development after cut stem inoculation by *M. phaseolina* (Amrate *et al*., 2019).

## CONCLUSION

It concluded that charcoal rot has been affecting soybean cultivation badly in most of the soybean growing districts of the state and could be one of the reasons for low yield. Varieties such as JS 95-60, JS 93-05 and JS 20-29 should be replaced with tolerant varieties in charcoal rot-sensitive zones. Continuous cultivation of Maize/Soybean - Chickpea may enhance *M. phaseolina* inoculum in soil and this may be avoided in charcoal rot prone districts. Variability was detected in isolates that could be utilized in further research programmes.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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