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Pawan K Amrate
Department of Plant Pathology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

MS Bhale
Department of Plant Pathology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

MK Shrivastava
Department of Plant Breeding and Genetics, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

Corresponding Author:
Pawan K Amrate
Department of Plant Pathology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India

Pre and post emergence mortality in soybean seedling by *Macrophomina phaseolina* isolates

Pawan K Amrate, MS Bhale and MK Shrivastava

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Abstract
Charcoal rot of soybean incited by *Macrophomina phaseolina* (Tassi) Goid [=*Rhizoctonia bataticola* (Taub.) Butler] is a serious disease causes considerable yield loss across the world. Present investigation was undertaken to determine the pathogenic potential of sixteen isolates isolated from charcoal rot affected samples of different districts of Madhya Pradesh, India. Three varieties i.e. JS 93-05, JS 20-29 and JS 95-60 were tested against each isolate by sick pot method. All sixteen isolates were found to be pathogenic and recorded pre and post emergence mortality significantly. Maximum pre-emergence (37.78%) and post emergence (20.63%) mortality was recorded from the isolate of Chhindwara (Mp-4$_{(CWA)}$). Significant highest mean percentage of pre-emergence (32.35%) and post emergence mortality (18.99%) was observed in JS 20-29 and JS 95-60, respectively. Based upon the average data of pre and post emergence mortality in three varieties (table 3), isolates from Chhindwara (Mp-4$_{(CWA)}$), Jabalpur (Mp-1$_{(JBP)}$), Hoshangabad (Mp-6$_{(HBD)}$) and Seoni (Mp-3$_{(SEO)}$) were found to be highly virulent(>25% mortality). Whereas, isolates from Umariya (Mp-12$_{(UMR)}$), Rajgarh (Mp-15$_{(RJG)}$) and Raisen (Mp-10$_{(RSN)}$) were least virulent (Up to 20% mortality).

Keywords: *Macrophomina phaseolina*, isolate, soybean, pre-emergence, post-emergence, mortality

Introduction
Soybean [*Glycine max* (L.) Merrill] is well recognized for high protein content and used in several food products, animal feed and processing industries. It is one of the most important oilseed crops in India. During 2016-17, the area, production and productivity of soybean in India was 11.18 Mha, 13.16 MT and 1177 kg/ha, respectively (Anonymous, 2018) [3]. Madhya Pradesh is a prime state for soybean production contributes more than fifty percentages of area and production. In India, soybean suffers with several biotic and abiotic stresses throughout the cropping season. It can be attacked by more than 100 pathogens (Sinclair, 1984) [15].
A fungus, *Macrophomina phaseolina* (Tassi) Goid [=*Rhizoctonia bataticola* (Taub.) Butler] causal agent of charcoal rot disease is one of most destructive pathogen survive in soil and have than 500 host (Kunwar *et al.*, 1986; Smith and Wyllie, 1999) [10, 16]. Existence of *M. phaseolina* in two asexual forms with broad host range maintains it survival better (Dhingra and Sinclair, 1978; Cloud and Rupe, 1988) [4, 5]. Charcoal rot of soybean has been categorized among important disease that causes considerable yield losses in top eight soybean producing countries including India (Wrather *et al*. 2010) [19]. During recent time, charcoal rot has emerged as one of the major constraints for soybean cultivation in Madhya Pradesh. It occurs regularly with moderate to severe form and affects most of the presently growing soybean varieties and important germplasm (Amrate *et al.*, 2018 & 2019) [1, 2].
Several researchers have been reported that *Macrophomina phaseolina* isolated from similar crop growing at different places exhibits variation for cultural characteristic as well as pathogenic potential (Prameela & Singh, 1998; Purkayastha *et al.*, 2014; Varma and Pathe, 2013; Manjunatha and Saifulla, 2018) [11, 13, 14, 18]. In view of increasing incidence of disease and nature of pathogen, an investigation was untaken to determine the most pathogenic isolate so that it could be utilized in genotypic resistance screening and other studies.

Materials and Methods
The causal agent of charcoal rot of soybean was isolated from disease affected sample of sixteen districts of Madhya Pradesh. Disease affected small pieces of tissue (tap root) showing

typical grayish blackening was first surface sterilized (by dipping in 2% sodium hypochlorite for 30 to 60 second) and then after, transferred to Potato Dextrose Agar (PDA) medium amended with streptomycin sulphate (figure 2). Thereafter, it was purified by hyphal tip transfer, and identified as *Macrophomina phaseolina* based upon typical cultural and microsclerotial characteristics (Dhingra and Sinclair, 1978; Gupta *et al.*, 2012) [5, 7]. The culture of all sixteen isolated from different districts were mass multiplied on rice grain (figure 2). Hundred gram of rice and 20 ml of water were mixed thoroughly in 250 ml conical flask and autoclaved at 121°C temperature and 15 lbs pressure for 15 minutes. Thereafter, it was inoculated with actively growing mycelia discs of *M. phaseolina* and incubated at temperature 26±1 °C for 21 days. Flasks were repeatedly shaken on every alternate day for proper mixing and multiplication of inoculum. Presence of numerous Microsclerotial body of the fungus was examined on colonized grain of rice under stereoscope microscope.

Surface sterilized (0.1% formalin) earthen pots were filled with approximately 1.0 kg sterile soil. Subsequently, 10 gram mass multiplied inoculum of each isolates was mixed in upper layer of soil in pot. Each isolates were tested against three varieties (JS 93-05, JS 95-60 and JS 20-29) with keeping three replications. Surface sterilized (2% sodium hypochlorite for 1 minute) 10 seeds of each variety were sown in infested pot and kept under poly house. Observation for emergence of seedling and post-emergence death were recorded at 8^{th} and 30^{th} days after sowing, respectively. Pre and post-emergence mortality of soybean seedlings were calculated.

Results and Discussion

The results revealed that all the isolates of *Macrophomina phaseolina* caused pre- emergence mortality in all three varieties of soybean (table 1 & figure 1). Percentage of pre-emergence (24.44 - 37.78%) mortality in all sixteen isolates was significantly higher in comparison to un-inoculated (16.67%). Maximum pre-emergence mortality of 37.78% was recorded from the isolate of Chhindwara (Mp-$4_{(CWA)}$) followed by 36.67% from Hoshangabad (Mp-$6_{(HBD)}$), 35.56% from Jabalpur (Mp-$1_{(JBP)}$) and 35.56% from Seoni (Mp-$3_{(SEO)}$). The isolate from Rajgarh district (Mp-$15_{(RJG)}$) resulted in least mortality of 24.44%. Emergence of seedlings was affected by all the isolates and significant highest mean percentage of pre-emergence mortality (32.35%) was observed in JS 20-29.

The isolates were also recorded significant post-emergence mortality over un-inoculated pots (table 2 and figure 1). Among the isolates, the percentage of post emergence mortality ranged between 5.95 (Mp-$12_{(UMR)}$) - 20.63 (Mp-$4_{(CWA)}$). The highest percentage of post-emergence mortality was observed from the isolate of Chhindwara (Mp-$4_{(CWA)}$) followed by Mp-$1_{(JBP)}$ (20.37%), Mp-$6_{(HBD)}$ (18.52%) and Mp-$2_{(NAR)}$ (18.32%), respectively and were statistically at par to each other. All three varieties were responded differently as recorded significant post-emergence mortality. JS 95-60 was found to be most affected as recorded the maximum mean post-emergence mortality (18.99%), followed by JS 93-05 (13.01%) and JS 20-29 (9.79%), respectively.

Based upon the average data of pre and post emergence mortality (%) caused by all sixteen isolates in three varieties (table 3), isolates from Chhindwara (Mp-$4_{(CWA)}$), Jabalpur (Mp-$1_{(JBP)}$), Hoshangabad (Mp-$6_{(HBD)}$) and Seoni (Mp-$3_{(SEO)}$) were found to be highly virulent (>25% mortality). Whereas isolates from Umariya (Mp-$12_{(UMR)}$), Rajgarh (Mp-$15_{(RJG)}$), Raisen (Mp-$10_{(RSN)}$) were least virulent (Up to 20% mortality). Rest other isolates were showed moderate virulence (>20-25% mortality). In varieties, JS 95-60 was more susceptible as recorded highest (23.41%) mortality in comparison to JS 20-29 (21.07%) and JS 93-05 (20.62%).

In preset investigation, all the sixteen isolates of *Macrophomina phaseolina* belongs to different districts of Madhya Pradesh exhibited pathogenic potential. Iqbal and Mukhtar (2014) [8] also conducted detailed studies on 65 isolates of *Macrophomina phaseolina* collected from divers agroecological regions, and indicated significant differences in pathogenicity and classified the isolates into highly virulent (8), least virulent (10) and moderately virulent against mungbean cultivars. Gade *et al.* (2018) [6] also reported all 40 isolates, collected from different soybean growing areas of India, were varied significantly for pathogenic potential and two were highly pathogenic with >70% mortality. Likewise, several other researcher also found significant variation in pathogenic potential among isolates of *Macrophomina phaseolina* (*R. bataticola*) in different crops (Kanchan and Biswas, 2009; Subramanian *et al.* 2011; Mohan and Balabaskar, 2012) [9, 12, 17].

Table 1: Pre-emergence mortality of soybean seedlings in artificially inoculated soil by isolates of *M. phaseolina*

Isolates	District	Pre-emergence mortality (%)			
		JS 93-05	JS 20-29	JS 95-60	Mean
Mp-$1_{(JBP)}$	Jabalpur	36.67	36.67	33.33	35.56
Mp-$2_{(NAR)}$	Narsingpur	26.67	33.33	26.67	28.89
Mp-$3_{(SEO)}$	Seoni	33.33	40.00	33.33	35.56
Mp-$4_{(CWA)}$	Chhindwara	36.67	40.00	36.67	37.78
Mp-$5_{(BET)}$	Betul	30.00	33.33	33.33	32.22
Mp-$6_{(HBD)}$	Hoshangabad	33.33	43.33	33.33	36.67
Mp-$7_{(HRD)}$	Harda	26.67	36.67	23.33	28.89
Mp-$8_{(DMH)}$	Damoh	23.33	33.33	30.00	28.89
Mp-$9_{(SGR)}$	Sagar	33.33	40.00	33.33	35.56
Mp-$10_{(RSN)}$	Raisen	26.67	30.00	20.00	25.56
Mp-$11_{(VDS)}$	Vidisha	23.33	33.33	26.67	27.78
Mp-$12_{(UMR)}$	Umaria	23.33	30.00	23.33	25.56
Mp-$13_{(SHD)}$	Shahdol	26.67	23.33	30.00	26.67
Mp-$14_{(SHR)}$	Sehore	30.00	26.67	30.00	28.89
Mp-$15_{(RJG)}$	Rajgarh	26.67	23.33	23.33	24.44
Mp-$16_{(UJN)}$	Ujjain	30.00	26.67	20.00	25.56
Un inoculated	-	13.33	20.00	16.67	16.67
Mean		28.24	32.35	27.84	-
CD (p=0.05)		Variety=2.74, Isolate = 6.53, Variety X Isolate = N/A			
SE (m)		Variety= 0.97, Isolate = 2.33, Variety X Isolate = 4.02			

Observation on 8^{th} days after sowing

Table 2: Post-emergence mortality of soybean seedlings in artificially inoculated soil by isolates of *M. phaseolina*

Isolates	District	Post-emergence mortality (%)			
		JS 93-05	JS 20-29	JS 95-60	Mean
Mp-1(JBP)	Jabalpur	20.63	15.87	24.60	20.37
Mp-2(NAR)	Narsingpur	22.02	10.32	22.62	18.32
Mp-3(SEO)	Seoni	30.16	11.11	15.87	19.05
Mp-4(CWA)	Chhindwara	15.87	9.52	36.51	20.63
Mp-5(BET)	Betul	0.00	11.11	34.92	15.34
Mp-6(HBD)	Hoshangabad	24.60	5.56	25.40	18.52
Mp-7(HRD)	Harda	14.29	16.67	17.26	16.07
Mp-8(DMH)	Damoh	0.00	0.00	25.00	8.33
Mp-9(SGR)	Sagar	15.87	16.19	0.00	10.69
Mp-10(RSN)	Raisen	0.00	0.00	29.17	9.72
Mp-11(VDS)	Vidisha	13.69	11.11	27.98	17.59
Mp-12(UMR)	Umaria	0.00	9.52	8.33	5.95
Mp-13(SHD)	Shahdol	13.10	22.02	19.05	18.06
Mp-14(SHR)	Sehore	26.79	0.00	13.89	13.56
Mp-15(RJG)	Rajgarh	0.00	8.93	18.45	9.13
Mp-16(UJN)	Ujjain	24.21	18.45	3.70	15.45
Un inoculated	-	0.00	0.00	0.00	0.00
Mean		13.01	9.79	18.99	-
CD (p=0.05)		Variety=4.92, Isolate =11.73, Variety X Isolate = 20.32			
SE (m)		Variety=1.75, Isolate =4.17, Variety X Isolate =7.23			

Observation on 30th days after sowing

Table 3: Pooled Pre and post emergence mortality of soybean seedlings in artificially inoculated soil by isolates of *M. phaseolina*

Isolates	District	Pre and post emergence mortality (%)			Average (Overall)
		JS 93-05	JS 20-29	JS 95-60	
Mp-1(JBP)	Jabalpur	28.65	26.27	28.97	27.96
Mp-2(NAR)	Narsingpur	24.35	21.83	24.64	23.60
Mp-3(SEO)	Seoni	31.75	25.56	24.60	27.30
Mp-4(CWA)	Chhindwara	26.27	24.76	36.59	29.21
Mp-5(BET)	Betul	15.00	22.22	34.13	23.78
Mp-6(HBD)	Hoshangabad	28.97	24.44	29.37	27.59
Mp-7(HRD)	Harda	20.48	26.67	20.30	22.48
Mp-8(DMH)	Damoh	11.67	16.67	27.50	18.61
Mp-9(SGR)	Sagar	24.60	28.10	16.67	23.12
Mp-10(RSN)	Raisen	13.33	15.00	24.58	17.64
Mp-11(VDS)	Vidisha	18.51	22.22	27.32	22.69
Mp-12(UMR)	Umaria	11.67	19.76	15.83	15.75
Mp-13(SHD)	Shahdol	19.88	22.68	24.52	22.36
Mp-14(SHR)	Sehore	28.39	13.33	21.94	21.22
Mp-15(RJG)	Rajgarh	13.33	16.13	20.89	16.79
Mp-16(UJN)	Ujjain	27.10	22.56	11.85	20.50
Un inoculated	-	6.67	10.00	8.33	8.33
Average		20.62	21.07	23.41	21.70

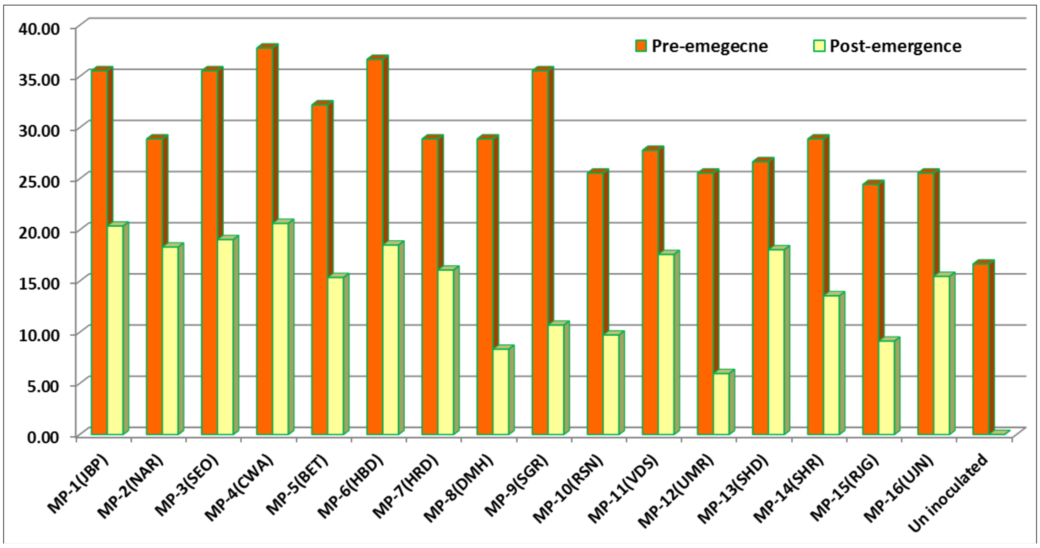

Fig 1: Average Pre & Post-emergence mortality of seedlings of soybean varieties due to different isolates of *M. phaseolina* in artificially inoculated pot soil

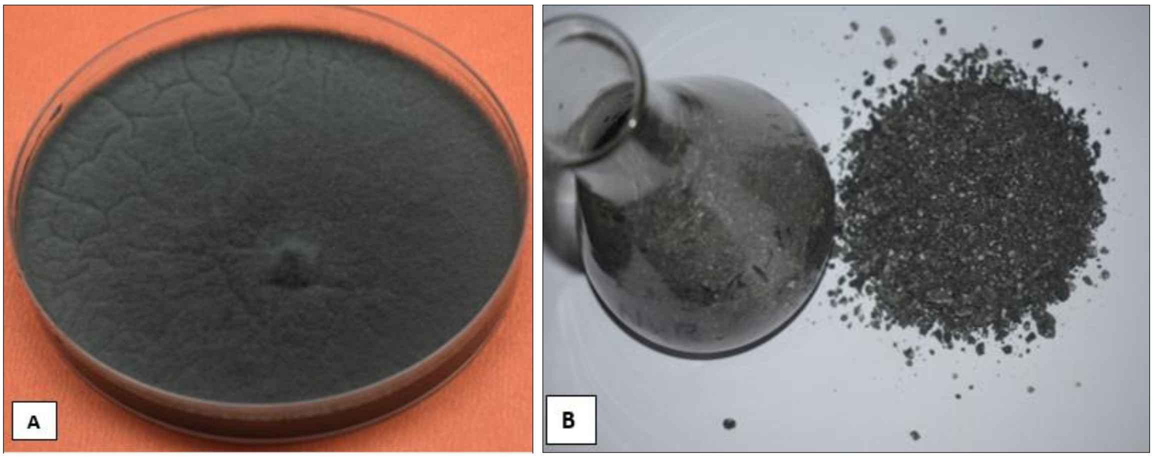

Fig 2: Close up view of 7 days old culture of *Macrophomina phaseolina* on Potato Dextrose Agar plate (A) and 21 days mass multiplied culture on Rice grain (B), respectively

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