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Variability of *Macrophomina phaseolina* (Tassi.) Goid. the causal organism of root rot of sesame and its management

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Abstract
Ten isolates of *Macrophomina phaseolina* (Tassi) Goid. (sclerotial stage: *Rhizoctonia bataticola* (Taub) Butler) collected from different major sesame growing areas of Telangana, Karnataka and Andhra Pradesh were studied for their variability on morphological, cultural and pathogenic characteristics. The hyphal cell size varied from 13.75 x 3.75 µm (Mp 9) to 31.39 x 4.5 µm (Mp 5), size of sclerotia varied from 82.41 x 58.90 µm (Mp 6) to 135.18 x 101.88 µm (Mp 8). Based on shape of sclerotia the isolates were classified in to three groups. irregular, round and ovoid groups. The number of sclerotia per microscopic field at 10x varied from 14 (Mp 2) to 42 (Mp 7). There was no significant difference in the colony growth rate recorded among the isolates of *M. phaseolina* which varied from 7.66 to 8.65 mm, Isolates categorized into three groups *viz.*, partially submerged, submerged, fluffy on the basis of colony texture and colony colour varied from black, grey and light grey colour. Based on the disease incidence isolates were categorized into two categories *viz.*, virulent (2 isolates) and highly virulent (8 isolates).

Keywords: Variability, root rot, *Macrophomina phaseolina*, sclerotia

Introduction
The crop is cultivated in a wide range of atmospheres, extending from semi-arid tropics and subtropics to temperate areas of the world (Raikwar and Srivastava, 2013). India is the leading producer of sesame seeds in the world (FAO STAT, 2014) [8]. In India, sesame crop occupies an area of 1.77 Mha with production of 8.27 lakh tonnes and productivity of 426 kg ha^{-1} (Indiastat, 2014-15) and in Telangana it is cultivated over an area of 0.28 lakh ha with the production of 0.09 lakh tonnes and productivity of 304 kg ha^{-1} (Agricultural action plan 2015-16).
Sesame (*Sesamum indicum* L.) is under constant threat to many diseases *viz.*, charcoal rot/stem rot/root rot, powdery mildew, leaf blight, wilt, leaf spot, stem blight,, bacterial leaf spot and phyllody. Among these root rot/stem rot caused by *Macrophomina phaseolina* (Tassi.) Goid (= *Rhizcotonia bataticola*) is the most important disease of sesame in India (Chattopadhyay and Sastry, 1998) [5]. It has become a potential threat for the profitable cultivation especially in the changing warm climate and intensive farming situations (Saharan *et al.*, 2005) [18]. In view of the importance of the crop and disease management, the present work is planned to study the culture characteristics, morphological and pathogenic variability among the isolates of *Macrophomina phaseolina.*

Materials and Methods
Morphological and cultural characterization of *M. phaseolina*
Ten isolates of the pathogen were selected for morphological and cultural characteristics (Table 1). Since the root rot disease of sesame is a major problem in Karnataka and Andhra Pradesh apart from Telangana so representative disease samples were collected from these two states also and pathogen was isolated. These isolates were further used in variability studies. The mycelial discs of 5 mm diameter were cut from the edge of a three days old culture and transferred aseptically to 90 mm Petridish containing 15ml PDA. These plates were incubated at 28±1 °C. Each treatment was replicated thrice.

Table 1: Isolates of *M. phaseolina* from different sesame growing areas

Sl. No	Pathogen Isolated from	Isolate code
1	Polasa	Mp 1
2	Laxmipur	Mp 2
3	Morthad	Mp 3
4	Renjarla	Mp 4
5	Bussapur	Mp 5
6	Babapur	Mp 6
7	IIOR farm, Rajendranagar, Hyderabad	Mp 7
8	Narkhuda	Mp 8
9	Dharwad	Mp 9
10	Yelemanchili	Mp 10

Morphological variability

The slides of various isolates were prepared from 10 days old culture for studying morphological characteristics *viz*., size of hyphal length, hyphal width, mycelial colour, size of sclerotia, number of sclerotia, colour of sclerotia and shape of sclerotia were recorded after 10 days of incubation by using Mycaps image analyser.

Cultural variability

The colonies of isolates were characterized for growth rate at 72 h after incubation.Seven days old cultures were used to record texture, colour of the colony and presence or absence of aerial mycelium**.**

Pathogenic variability

For assessing the pathogenic variability of *M. phaseolina* isolates sick pot soil method (Salunkhe *et al.* 2014) [23] was followed. The potting mixture was prepared thoroughly mixing clay loam soil, sand and farm yard manure at 1:1:1 ratio. The inoculum of each isolate of *M. phaseolina* collected from different locations were separately mixed at five per cent level (w/w) with the sterilized soil filled in 30cm earthen pots ten days before sowing. Surface sterilized (using 0.1% mercuric chloride solution for 30 sec. followed by two washings in sterile water) sesame seeds were sown at 30 seeds per pot. Three replications were maintained in a completely randomized design and the sesame cultivar VRI-I was used in this study. The pots were maintained in glass house with regular, judicious and uniform watering. The data on germination was taken ten DAS and root rot incidence was recorded at 35 DAS, the per cent disease incidence was calculated and isolates are grouped as Least virulent, Virulent, Highly Virulent according to per cent disease incidence (Gupta *et al.* 2012) [9] as mentioned below.

Isolate category	**Per cent mortality**
Least virulent	< 20 Per cent
Virulent	21-50 Per cent
Highly virulent	> 51 Per cent

Mass multiplication of the pathogen

The inoculum of the test pathogen, *M. phaseolina* maintained on agar slants was further multipliedon sorghum grains. One hundred grams of sorghums eeds were washed thoroughly in tap water and soaked overnight in 250 ml conical flasks with addition of 20 ml of 4 per cent dextrose. The flasks were then autoclaved for 20 min at 15 lbs. After cooling the flasks at room temperature they were shaken well to separate the sterilized grains and were inoculated with 2-3 discs of 4 day old culture of *M. phaseolina* and incubated at 28 ± 1°C for seven days in BOD incubator. After seven days, the inoculum was mixed with sterilized soil in pots at five per cent level (w/w).

Results and Discussion

Morphological variability in *M.phaseolina*

Variability in the cultural and morphological characteristics of ten isolates of *M. phaseolina* were studied on potato dextrose agar medium. The colony growth rate of the *M. phaseolina* isolates was measured at 72h after inoculation. The size of the hyphal cell and sclerotia was measured using Mycaps image analyzer software at 40x objective of the microscope. Observations on various cultural and morphological characteristics were recorded as described in material and methods and the results obtained are presented in Table 2 and Table 3.

Hyphal cell

The hyphal cell size varied from 13.75 x 3.75 µm (Mp 9) to 31.39 x 4.5 µm (Mp 5). Ratio between length and width of hyphal cell varied from 2.29 (Mp 8) to 6.97 (Mp 5). Significant variation was observed among the isolates regarding cell size, length and width of the hyphal cell. The isolates Mp 1 and Mp 3, Mp 4 and Mp 6 were on par with each other.

Sclerotial size

The data presented in Table 2. revealed that on the basis of microscopic observations the size of sclerotia varied from 82.41 x 58.90 µm (Mp 6) to 135.18 x 101.88 µm (Mp 8). The isolates were classified in to three groups, *viz.*, small size (4000-5000 μm^2) medium (5001-6000 μm^2) and large size (more than 6000 μm^2). Of the 10 isolates, one isolate (Mp 6) was categorized in small, 2 isolates (Mp 3 and Mp 10) were in medium and 8 isolates (Mp 1, Mp 2, Mp 4, Mp 5, Mp 7, Mp 8, Mp 9) in large sized sclerotia category based on classification given by Varma and Pathe (2013) [24] details are depicted in Table 2.1.

These findings are in confirmation with the work of Mandal *et al.* (1998) [14] who reported that the sclerotial size ranged between 66.14 - 128.25 um. Dhingra and Sinclair (1972) [6] obseraved variation in sclerotial size (173.88 - 188.33 x 51.09 - 155.90 um), Sobti and Sharma (1992) [21] reported sclerotial size of 60 - 165 x 57 - 114 um.

Sclerotial shape

The isolates were categorized into irregular, round and ovoid groups based on shape of sclerotia (fig.1). Irregular shaped sclerotia were observed in 3 isolates (Mp 7, Mp 8, Mp 9) while ovoid shaped sclerotia were observed in 6 isolates (Mp 1, Mp 2, Mp 4, Mp 5, Mp 6, Mp 10). Round shape of sclerotia was observed in one isolate (Mp 3). Details of categorization is mentioned in Table 2.2.

These results are in agreement with those findings reported by Mandal *et al.* (1998) [14], who observed irregular and round to elongate sclerotial shape. Gupta *et al.* (2012) [9] categorized the isolates into two groups *viz.*, oblong shape with irregular edges and round with regular edges based on sclerotial morphology.

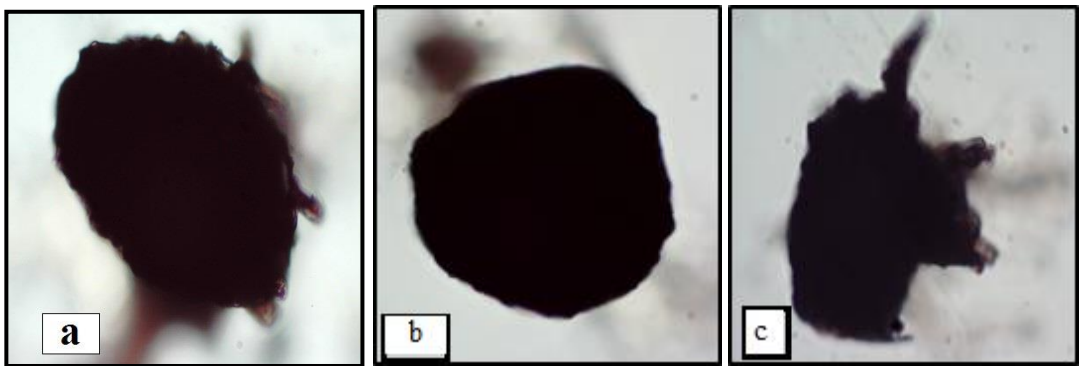

Fig 1: variability in scelotial shape a: Oblong, b: Round c: Irregular shape.

Sclerotial intensity

The number of sclerotia per microscopic field when observed through 10x objective varied from 14 (Mp 2) to 42 (Mp 7). On the basis of number of sclerotia per microscopic field the isolates were grouped (Table 2) in to three groups *viz.*, sparse (2 isolates), medium (2 isolates) and abundant (6 isolates). Details of categorization is depicted in Table 2.3.

Similar results were obtained by Hooda and Grover (1982) [10] who reported that Hyderabad isolate produced highest number of sclerotia (180.3 sclerotia/ 9 mm. disc and 52/ microscopic 10 x field) whereas Coimbatore isolate produced minimum number of sclerotia (169 sclerotia). Varma and Pathe (2013) [24] observed that the number of sclerotia ranged from 9.7 to 22.2 and 40 per cent of the isolates were having sparse number of sclerotia.

Cultural variability in *M. phaseolina*

Colony growth rate

There was no significant difference in the colony growth rate recorded among the isolates of *M. phaseolina* which varied from 7.66 to 8.65 mm (Table 3). Isolate Mp 7, showed highest colony growth (7.66 cm) while the least colony diameter was observed with the isolate Mp 3 (8.65 cm) at 72 hours after incubation.

These results are in agreement with Varma and Pathe (2013) [24] who observed that in 22 isolates of *R. bataticola* isolates did not differ in the type of mycelial growth and growth rate on potato dextrose agar medium.

Colony colour

Based on visual observation on colony colour, the cultures were divided into three groups. Black, grey and light grey coloured colonies (fig.2). Three isolates produced black coloured colony. while three isolates had grey coloured colony and four isolates were found to have light grey colour colony (Table 3).

Similar obseravations were also made by previous workers Shekhar *et al.* (2006) [20] on the basis of colony colour, divided seven isolates into four groups *viz.*, grayish white, blackish gray, dark black in centre periphery cremish and cottony white colour. Mohanapriya *et al.*, (2017) [22] also reported that all the ten isolates of the root rot pathogen *M. phaseolina* produced white, whitish grey, grey, black scanty to profusely aerial mycelial growth on Potato Dextrose Agar (PDA) medium.

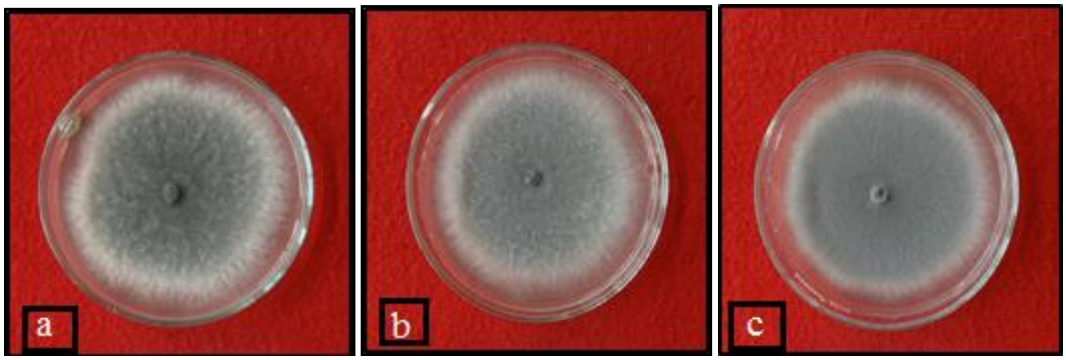

Fig 2: Variability in colony coloura: Black, b:Grey, c: Light grey colour

Colony texture

Isolates categorized into three groups *viz.*, partially submerged, submerged, fluffy on the basis of colony texture (fig 3). Of the 10 isolates eight isolates (Mp 1, Mp 2, Mp 5, Mp 6, Mp 7, Mp 8, Mp 9, Mp 10) produced partially Submerged colony while one isolate (Mp 4) had Submerged texture and one isolate (Mp 3) had produced fluffy growth (Table 3).

The colony texture of partially submerged was observed in Mp 1, Mp 2, Mp 5, Mp 6, Mp 7, Mp 8, Mp 9, Mp 10 isolates. Submerged texture was found in Mp 4, and fluffy growth in Mp 3 isolate. Details of grouping of isolates is given in Table 3.1.

Similar observations were made by Varma and Pathe (2013) [24] who classified 22 isolates of *R. bataticola* on the basis of type of growth *viz.*, fluffy and submerged, sixteen isolates were grouped in fluffy growth and six in sub merged group. Byadgi and Hedge (1985) [4] reported fluffy, submersed, dark brown mycelium and appressed light brown mycelial growth on different isolates of *R. bataticola*. Other workers have also reported the variation in type of growth of *R. bataticola* isolates (Sobti and Sharma, 1992, Ratnoo *et al.* 1997, Hooda and Grover, 1982) [21, 17, 10] grouped them in to 4-5 groups.

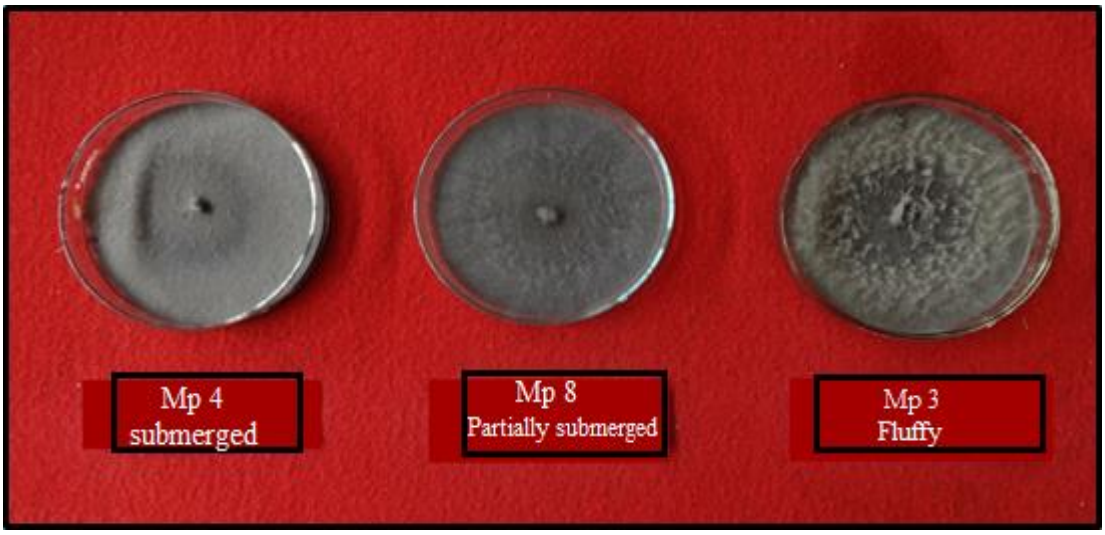

Fig 3: Variability in colony texture*viz.*, submerged, partially submerged, fluffy growth of the colony

Pathogenic variability in *M. phaseolina*

Variation in pathogenicity was found among 10 isolates of the *M. phaseolina* when inoculated on sesame cultivar VRI-I. Eight isolates *viz.*, Mp 1, Mp 2, Mp 4, Mp 5, Mp 6, Mp 7, Mp 8, and Mp 9 were found to be highly virulent while two isolates Mp 3, Mp 10 as virulent.

All the isolates of *M. phaseolina* induced variable reaction on susceptible sesame cultivar VRI-I in soil infestation technique (fig.4). Based on the disease incidence isolates were categorized into virulent (2 isolates) and highly virulent (8 isolates) as depicted in table 4.

Percent disease incidence varied from 44.35% to 73.33%. Accordingly eight isolates (Mp 1, Mp 2, Mp 4, Mp 5, Mp 6, Mp 7, Mp 8, and Mp 9) were categorized as highly virulent as the percent disease incidence was > 51 Per cent however, only two isolates (isolate Mp 3 and Mp 10) grouped as virulent type which recorded 21-50 Per cent disease incidence.

The perusal of the data indicated that among the isolates, the isolate Mp 6 from Babapur of Nirmal dostrict exhibited highest virulence with disease incidence of 73.33% followed by Renjarla isolate Mp 4 (70.47%) from of Nizamabad district, Mp 1 (66.66%), Mp 2 (63.64%), Mp 9 (60.00%), Mp 5 (59.82%), Mp 7 (57.33%), and Mp 8 (53.33%), However, minimum percent disease incidence (44.35%) was exhibited by Mp 10 isolated from Yelemanchili and categorized to be as virulent on cultivar VRI-I.

Similar variation in the pathogenicity of different isolates were reported (Shekhar *et al.*, 2006) [20] and based on the degree of aggressiveness *M. phaseolina* isolates weregrouped as most virulent and least virulent isolates. Three groups *i.e.* virulent, intermediate and mild on the basis of pathogenicity has been reported by Byadgi and Hegde (1985) [4]. Other workers have also reported the differential reaction by the isolates of *R. bataticola* in chickpea (Ratnoo *et al.*, 1997 and Monga and Sheo Raj, 1994) [17]. and found considerable variation among the isolates.

Sobti and Sharma (1992) [21] also recorded 13 to 63 per cent root rot incidence of groundnut with different isolates of *R. bataticola.* Ratnoo *et al*. (1997) [17] reported the pathogenic variation in the isolates of *M.phaseolina* from different cowpea growing areas of Udaipur. The pathogenic variability of this fungus has been described in different host plants such as soybean and sunflower (Dhingra and Sinclair, 1978; Jimenez *et al.*, 1983) [7], and variation in pathogenicity assumed to be due to mutation, hyphal fusion and mitotic recombination. The rapid growth or spread of the mycelia and the abundant occurrence of sclerotia due to conductive environmental conditions may also have caused variation (Jimenez *et al.*, 1983) [12].

Table 2: Variability in morphological characteristics of *Macrophomina phaseolina* isolates

Sl. No	Isolate Code	Hyphal cell			Sclerotia					
		Length x Width (µm)		length : width ratio of hyphal cell	Length x Width (µm)		Area (µm^2)	Intensity per 10x microscopic field	Shape	colour
		Length (µm)	Width (µm)		Length (µm)	Width (µm)				
1	Mp 1	24.47	5.50	4.44	104.77	86.81	9095.08	18	Ovoid	Black
2	Mp 2	27.87	8.72	3.19	96.61	74.42	7189.71	14	Ovoid	Black
3	Mp 3	23.34	6.35	3.67	97.38	59.80	5823.32	15	Round	Black
4	Mp 4	24.14	6.18	3.9	96.48	80.79	7794.61	23	Ovoid	Black
5	Mp 5	31.39	4.5	6.97	110.58	89.51	9898.01	19	Ovoid	Black
6	Mp 6	24.49	5.87	4.17	82.41	58.90	4853.95	35	Ovoid	Black
7	Mp 7	26.47	5.94	4.45	91.13	71.56	6521.26	42	Irregular	Black
8	Mp 8	21.46	9.34	2.29	135.18	101.88	13772.1	27	Irregular	Black
9	Mp 9	13.74	3.75	3.6	92.96	74.44	6919.94	29	Irregular	Black
10	Mp 10	27.53	4.62	5.9	82.97	69.12	5734.88	26	Ovoid	Black
C.D.		0.86	0.51	0.34	1.71	0.69	171.53	1.11		
SE(m)		0.29	0.17	0.11	0.58	0.23	57.74	0.37		
C.V.		2.04	4.94	4.70	1.01	0.52	1.28	2.62		

Table 2.1 Grouping of isolates of *Macrophomina phaseolina* on the basis of sclerotial size Varma and Pathe (2013)

Sl. No	Characters	Isolates
1	Small (4000-5000 µm^2)	Mp 6
2	Medium (5001-6000 µm^2)	Mp 3, Mp 10
3	Large (more than 6000 µm^2)	Mp 1, Mp 2, Mp 4, Mp 5, Mp 7, Mp 8, Mp 9,

Table 2.2: Grouping of isolates of *Macrophomina phaseolina* on the basis of intensity of the sclerotia

Sl. No	Characters	Isolates
1	Sparse (9 -15)	Mp 2, Mp 3
2	medium (15.1-21.0)	Mp 1, Mp 5
3	Abundant (more than 21.0)	Mp 4, Mp 6, Mp 7, Mp 8, Mp 9, Mp 10

Table 2.3: Grouping of isolates of *Macrophomina phaseolina* on the basis of shape of the sclerotia

Sl. No	Characters	Isolates
1	Round	Mp 3
2	Ovoid	Mp 1, Mp 2, Mp 4, Mp 5, Mp 6, Mp 10
3	Irregular	Mp 7, Mp 8, Mp 9

Table 3: Variability in cultural characteristics of *Macrophomina phaseolina* isolates

Sl. No	Isolate Code	Growth Rate (cm)	Pigmentation	Colony texture
1	Mp 1	7.50	Black	PS
2	Mp 2	7.20	Light Grey	PS
3	Mp 3	8.70	Light Grey	F
4	Mp 4	8.57	Light Grey	S
5	Mp 5	8.00	Grey	PS
6	Mp 6	7.90	Light Grey	PS
7	Mp 7	6.80	Black	PS
8	Mp 8	7.60	Grey	PS
9	Mp 9	7.80	Grey	PS
10	Mp 10	8.40	Black	PS
C.D.		0.34		
SE(m)		0.11		
C.V.		2.55		

F: Fluffy PS: Partially submerged S: Submerged

Table 3.1. Grouping of isolates of *R. bataticola* on the basis of mycelial growth

Sl. No	Characters	Isolates
1	Submerged	Mp 4
2	Partially Submerged	Mp 1, Mp 2, Mp 5, Mp 6, Mp 7, Mp 8, Mp 9, Mp 10
3	Fluffy	Mp 3

Table 4: Pathogenic Variability of *Macrophomina phaseolina* isolates

Sl. No	Isolate Code	Percent Disease Incidence (%)	Virulence category
1	Mp 1	66.66 (54.71) *	Highly Virulent
2	Mp 2	63.64 (52.90)	Highly Virulent
3	Mp 3	46.00 (42.68)	Virulent
4	Mp 4	70.47 (57.07)	Highly Virulent
5	Mp 5	59.82 (50.64)	Highly Virulent
6	Mp 6	73.33 (58.89)	Highly Virulent
7	Mp 7	57.31 (49.18)	Highly Virulent
8	Mp 8	53.33 (46.89)	Highly Virulent
9	Mp 9	60.00 (50.75)	Highly Virulent
10	Mp 10	44.35 (41.73)	Virulent
C.D.		2.55	
SE(m)		0.86	
C.V.		2.95	

* Values in the parentheses are angular transformed and are the means of three replications

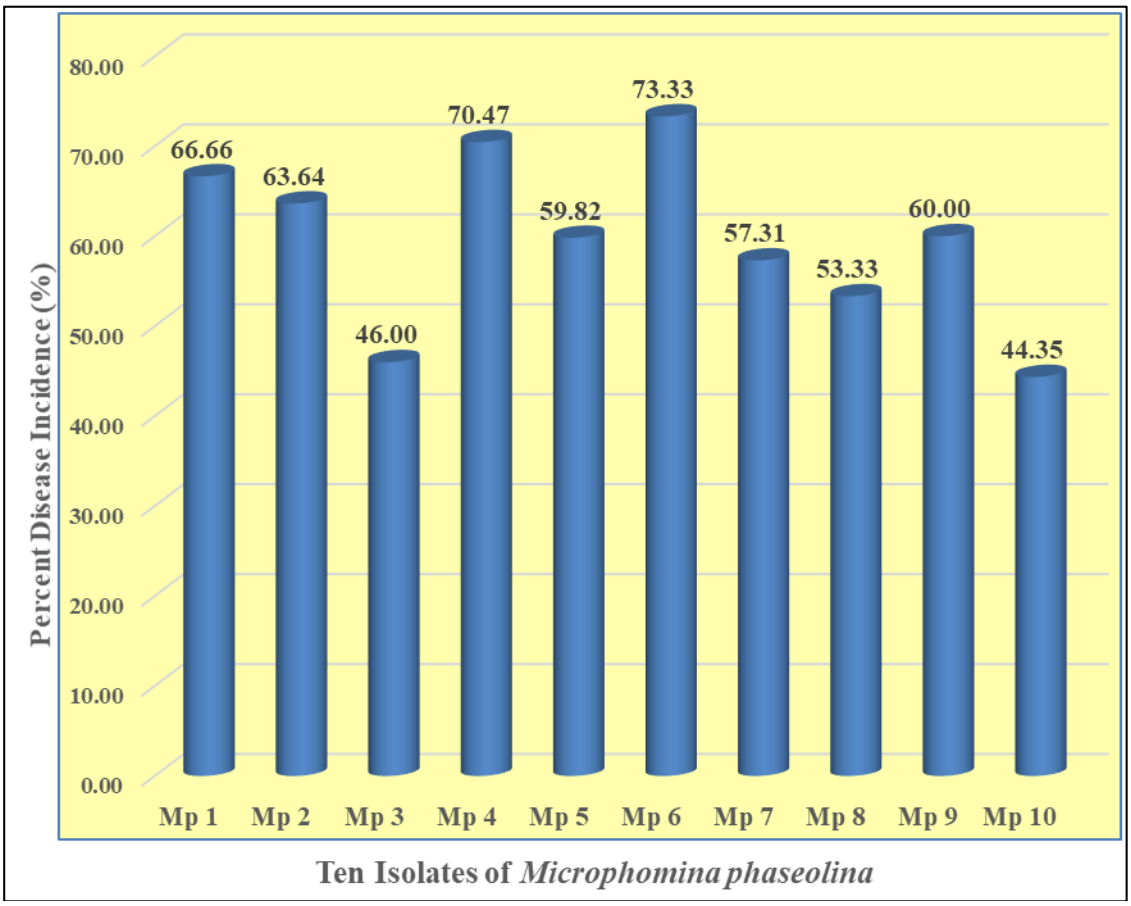

Fig 4: Variability in pathogenicity of ten *Macreophomina phaseolina* isolate

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