Interpreting Variability among *Macrophominaphaseolina* isolates causing dry root rot of clusterbean.

Authors:

Priyanka[1],R.S.Sharma[2],A.C.Mathur[3],R. K.Bagri[4],SushilaChoudhary[5], KiranHingonia[6]

Authors Detail:

1. AssistantProfessor,PlantPathology,CollegeofAgriculture,Sumerpur,AU,Jodhpur,302069
2. AssistantProfessor,DivisionofPlantPathology,RARI,Durgapura,SKNAU,Jobner
3. ProfessorDivisionsofPlantPathology,RARI,Durgapura, SKNAU, Jobner
4. AssociateProfessorDivisionsofPlantPathology,RARI,Durgapura,SKNAU,Jobner
5. PhDscholars,DivisionofPlant Pathology, RARI,Durgapura,SKNAU,Jobner
6. AssistantProfessor, Agronomy,CollegeofAgriculture,Sumerpur,AU,Jodhpur,302069

Corresponding Author:
Dr. Priyanka, Assistant Professor, Plant Pathology, College of Agriculture, Sumerpur, AU, Jodhpur, 306902
Mob. No. : +91 8306141688
Email: pri.poonia93@gmail.com

Abstract:-

Background: Clusterbean, eminently known as guar has been figured as a high-valued cash crop within the arid and semi-arid regions due to its drought robustness and multiple uses and it has occupied a special place in the commercial scene due to its gum. Dry root rot incited by *Macrophominaphaseolina*, a significant threat to clusterbean production because clusterbean is generally raised under moisture stress conditions and high temperature, which is conducive to developing dry root rot disease.

Methods: In the present field-laboratory investigation during 2019-21,twelve isolates of *M. phaseolina* collected from different agro climatic zone of Rajasthan during survey were studied for their cultural, morphological, and pathological variability.

Conclusion: Results of the present investigation concluded that regardless of their geographic origins, all the isolates showed considerable variation in colony colour, type, aerial mycelial, branching pattern, radial growth, shape, size, colour and number of sclerotia and virulence. Mp-BKN also found highly pathogenic on all tested five varieties with 69.95 per cent mean disease incidence and Mp-UDZ isolate was least virulent on all tested five varieties with minimum mean disease incidence (31.61%). Among the tested culture media all the isolates attained maximum mycelial growth of 85.20 mm on Potato Dextrose Agar

(PDA).The present investigations on the morphological, cultural and pathogenic variations in various isolates of *M. phaseolina*willbe considered important in disease management systems and useful in breeding programmes of cultivars resistant to dry root rot.

Key words: Clusterbean, *M. phaseolina,* Morphological, Cultural, Pathogenic, Variability

Introduction

[*Cyamopsistetragonoloba*(L.)], eminently known as guar, is a deep-rooted annuallegumecropoffamily*Leguminosae*(*Fabaceae*)knownforitsdroughtandhigh-temperature tolerance (Kumar and Rodge, 2012). It is figured as a high-valued cash cropwithin the arid and semi-arid regions due to its drought robustness and multiple uses andhas occupied a special place in the commercial scene due to its gum. Globally, India is thepreeminent clusterbean producing country and covering about 80 per cent of production.The total area, production and productivity of the clusterbean in India was 39.36 lakhhectares,16.24lakhtonesand428.0kg/ha,respectively(Anonymous,2020).Theproduction of the clusterbean crop has been stagnantbecause of its cultivation underrainfed areas, marginal and sub-marginal lands, low soil fertility, andbiotic stresses.Among biotic stresses diseases, insects, nematodes, and parasitic weeds are account forsignificantcroplosses.Thesignificantdiseasesofclusterbeanare*Alternaria*blight,Anthracnose, Dry Root rot, Bacterial blight, and Powdery mildew. Among these diseases,dry root rot is incited by *Macrophominaphaseolina*(Tassi) Goid. has become a majorbiotic threat in several regions of the country and causes considerable economic yieldlosses. Because clusterbean is generally raised under moisture stress conditions and hightemperature, which is conducive to developing dry root rot disease, this disease was not ofmuch significance in clusterbean earlier; however, it has become a significant threat toclusterbean production nowadays due to altered weather conditions, mainly due to longerdroughtspells andtheformidablenatureofits pathogen.

*M. phaseolina*is a non-specialized fungus well known for its survival in seed, stubble, andsoil-bornenature,attackingabout500hostspeciesinmorethan100familiesofeconomically important crops throughout the world (Mihail*et al*., 1995, Purkayastha*et al.*,2006). *Macrophomina*known as a polyphagous pathogen causes dry root rot or charcoal rotdiseasein several economically important crops such as legumes and vegetables (Kaur*etal*., 2012; Kumar *et al*., 2017). It is a soil-borne fungus that survives in soil for prolongedperiods (Dhingra and Sinclair, 1978). Low soil moisture is reported to increase growth andenhance the survival of *M. phaseolina*in soil (Short *et al*., 1980). The threat of dry root rotof clusterbean and subsequent damage to the crop cultivation was felt menaces to study thisdisease.Due to the high degree of genetic variation in the pathogen, research is needed toimprovetheidentificationandcharacterizationofgeneticvariabilitywithintheirepidemiological andpathologicalniches.Abetterunderstandingofthevariabilitywithinthe pathogen population for traits that influence fitness and soil survival will undoubtedlylead to improved management strategies for *M. phaseolina*.There are several reports fromdifferent parts of the world that populations of *M. phaseolina*showed morphological andpathogenic(Jana*etal*.,2003,Meena*etal*.,2006)variations.Thereforethepresentinvestigationa ccountstoevaluatethemorphological,culturalandpathogenicvariabilityof *M.phaseolina*inciting ofdryrootrotofclusterbean.

MaterialsandMethods

The morphological and cultural variability of *M. phaseolina*isolatesMycelialcharacters

For studying variability in radial growth one mycelial disc (5mm diameter)from a seven days old culture of all isolates of *M. phaseolina*were transferred on 2 per centPDA in Petri dishes and incubated in the dark at 28±2^{0}C for 96h. Each treatment wasreplicated four times. The colony diameter or radial growth of colony was recorded at 24hafter incubation up to 96 h. On the fifth day, main characteristics of the colonies wererecorded (color of colony, texture, presence/absence of aerial mycelium, colony appereanceand branching pattern)with the help of calibrated microscope (10X × 10X). Finally,after72 hours on the basis of radial growth, the cultures were categorized as fast (>80 mm),medium(61-80mm)andslow (<60mm)growing(IqbalandMukhtar2014).

Mycelialdry weight

Twenty ml of Potato Dextrose Broth (PDB) was poured into 150 ml conicalflasksandweresterilizedat1.1kg/cm^{2}for20min.Theflaskswereinoculatedwithagar blocks (5 mm diam.) cut from actively growing margin of seven-day old culture of *M.phaseolina*.Treatmentswerereplicatedthrice.Theculturesbwerefilteredthroughpre-weighedWhatman no.42 filterpaperafterincubation forseven daysat28±2^{0}C. Themycelial mats were dried at 85^{0}C for 24 h to determine the mycelial dry weight yield. Theactual weight of dry fungal mycelium was calculated using the following formula (Arey,2010).Weightofmycelium=(Weightoffilterpaper+WeightofMycelium)–(Weightoffilterpaper)Basedonthemycelialdryweighttheculturesweregroupedintohigh,medium andlow(Ashraf*etal*.2017).

Sclerotialcharacters

Formeasuringsclerotialsize,slidesfrom7day-oldpureculturesof*M.phaseolina*isolates were prepared and examinedunder a microscope ocularmicrometer.The observations on sclerotial characters*viz*., number, shape, size, colour, numbers ofsclerotial bodies formed per microscopic field (10X × 10X) at three spots in a Petri platewere recorded.Finally,the diameterof the sclerotia was recordedusingamicrometer,shape and color of microsclerotia were recorded at40X. The numberof sclerotia permicroscopicfieldwas calculated(Table 1),(Hooda andGrover,1982).

Table1:Relative degreeofsclerotialformationin*M. phaseolina*isolates

Meannumberofsclerotiapermicroscopicfield	Category
0	Nil
1-20	Few
21-40	Several
Above40	Abundant

Effectofdifferentsolidmediaongrowthof *Macrophominaphaseolina*

The study of different solid media was undertaken to find out the superior media forthe mycelial growth of *M. phaseolina*. Five different solid media *viz*., Potato DextroseAgar (PDA), Czapek's (Dox) and oat meal agar, Richard's Agar medium and Host leafextract agar were used and compared for this purpose. The required quantity of the abovementioned solid medium was prepared and sterilized at 1.045 kg/cm^2 pressure for 20minutes. Sterilization of Petri dishes was done at 180°C for 2 h in a hot air oven. In eachPetri dish, 25 ml of medium was poured. Each treatment was replicated four times. EachPetri dish was inoculated with a mycelial bit of 5 mm diameter maintained on plain agar.TheinoculatedPetridisheswereincubatedat28±2°Ctemperature,andobservationsonmycelialgrowthwererecordedaccordingly.Themediawerepreparedaccordingtothestandardformulagive nbyRikerand Ricker (1936)and Ainsworthand Bisby(1967).

Pathogenicvariability

The pathogenicvariability of twelvevirulentisolates of *M. phaseolina*weretested on five clusterbean varieties*i.e.*RGC-986,RGC-1038,RGC-1055, RGC-1017and RGC-936 in earthen pots of 30 cm diameter. The clusterbean varieties used in thestudywereobtainedfromDivisionofPlantBreedingandGenetics,RajasthanAgricultural Research Institute, Durgapura Sri Karan Narendra Agriculture University,Jobner, Jaipur. Clusterbean seeds were sown after 72 hours of soil inoculation.Thesorghumgraininoculaofindividual*M.phaseolina*isolateswereaddedtosterilizedsoilat 20 g per pot and mixed thoroughly. In each pot ten healthy seeds of each clusterbeanvariety were sown keeping four replications for each variety. In case of control, healthyclusterbean seeds of five corresponding varieties were sown in uninoculated sterilizedsoil. The disease symptoms were observed periodically up to 90 days of sowing, and percentincidencewas calculated.

ResultsandDiscussion

Morphologicalandculturalvariability

Significantvariationshavebeenobservedinmorphologicalandcultural characteristicsinalltheisolates of*M.phaseolina.*

Radialgrowth

Results depicted in Table 2 showed significant differences among the collectedtwelveisolates on thebasis of radial growth. Thecolony diameterandgrowth wererecorded at 24h after incubation up to 96h. Isolates of *M. phaseolina*fungus categorizedinto three classes on the basis of complete radial mycelium growth. Radial growth of allthe isolates was recorded at the 72h, 96h and more than 96h interval and then classifiedinto 3 groups: fast (72h), medium (96h) and slow growing (>96h). Three isolates Mp-DPA,Mp-BKNandMp-CURwerecompletedtheirradialgrowthwithin72hofinoculation proving to be the fast growing and five isolates growing isolates *viz*., Mp-JU,Mp-JSM, Mp-JJN Mp-NGO and Mp-SIKR were completed their radial growth within 96hof inoculation and categorized as medium growing while four isolates *viz*., Mp-AWR,Mp-HMH,Mp-SNGR,Mp-UDZshowedtheminimumradialgrowthsanddidn'tcomplete the total radial growth even after96h of inoculation and hence were rated asslowgrowing.Thegrowth rate of colony was recorded at48h afterincubation.Thegrowth speed of colony of twelve isolates ranged from 0.75 (Mp-UDZ) to 1.25 mm/hr(Mp-BKN)with1.00mm/hinaverage.

Colonycolourand texture

On the basis of visual observation results of present investigation depicted in Table 1andPlate 1 showed considerable variation among cultural and morphological variability inmycelial growth of the *M. phaseolina*isolates and on the basis of colony colour theseisolates were divided in three fractions, (i) black which include Mp-BKN (dark black),Mp-AWR(Black),Mp-CUR(Black),Mp-DPA(greyishblack),Mp-HMH(grayishblack), Mp-JSM (black), Mp-SIKR (greyish black) and Mp-SGNR (Greyish black), (ii)whitish colony colour of Mp-JJN (greyish white) and Mp-NGO (creamy white), Mp-UDZ(grayish white) and (iii) brown colour colony of Mp-JU (light brown). On the basis ofaerial mycelium production characteristics, *M. phaseolina*isolates were categorized inthree category, first high aerial mycelium growth (+++) was noticed in Mp-BKN, Mp-DPA, Mp-JJN and Mp- SIKR isolates, second average aerial mycelium growth(++), whichwas recorded in Mp-CUR, Mp-HMH, Mp-JU, Mp-JSM, Mp-NGO and Mp-SNGR isolatesand the third one was poor aerial mycelium growth (+) was recorded in Mp-AWR andMp-UDZisolates.

Collected isolates were varied in colony texture and based on colony texture theseisolates were categorizedinto three groups *viz.*, appressed,fluffy and partially fluffy.Among the collected isolates, four isolates *viz.*, Mp-BKN, Mp-HMH, Mp-JSM and Mp-NGO produced appressed colony while three isolates *viz.*, Mp-DPA, Mp-Mp-JJN and Mp-SIKR had fluffy texture colony and remaining five isolates, namely Mp-AWR, Mp-Mp-CUR,Mp-JU,Mp-SNGR andMp-UDZpartiallyfluffytexturetype colony.

Plate1:Colonycolorofdifferent*M.phaseolina*isolates

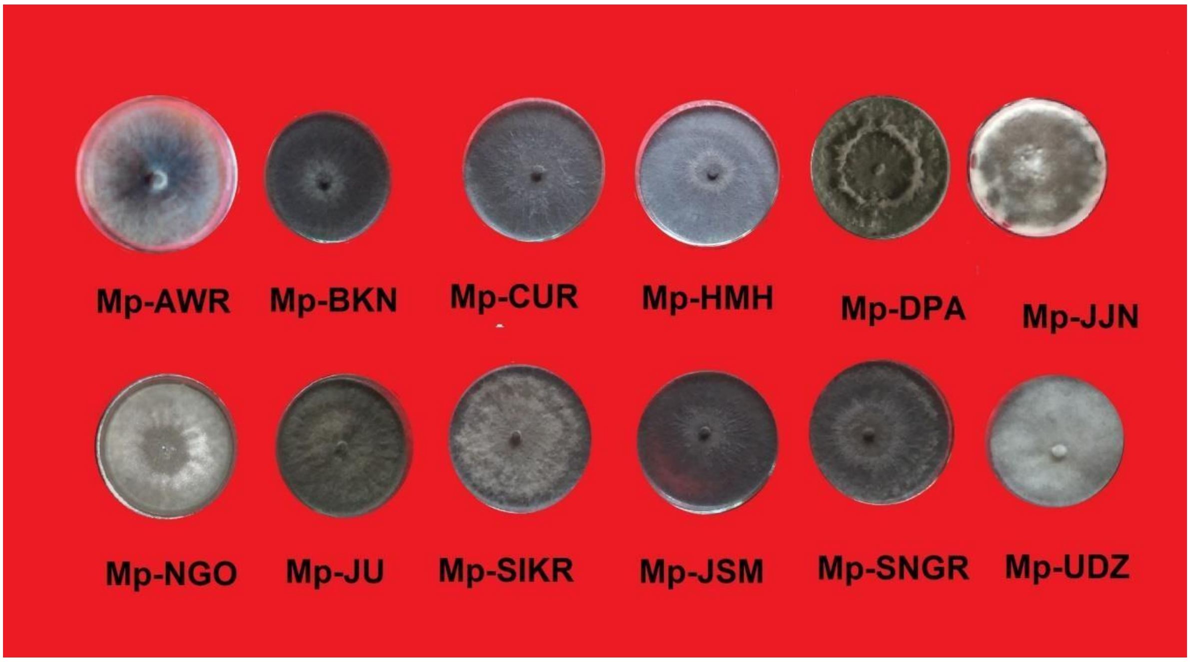

Table1Variationincolonycolour, texture,aerialmyceliumformation

Isolates	Colourofcolony(Reverse)	ColonyColor	Colonytexture	Aerialmycelium	BranchingPattern
Mp-AWR	Black	Black	Partiallyfluffy	+	Rightangle
Mp-BKN	Black	Darkblack	Appressed	+++	Rightangle
Mp-CUR	Black	Black	Partiallyfluffy	++	Rightangle
Mp-DPA	Black	Greyishblack	Fluffy	+++	Rightangle
Mp-HMH	Black	Greyishblack	Appressed	++	Rightangle
Mp-JJN	Black	Greyishwhite	Fluffy	+++	Right angle
Mp-JSM	Black	Black	Appressed	++	Rightangle
Mp-JU	Black	Lightbrown	Partiallyfluffy	++	Rightangle
Mp-NGO	Black	Creamywhite	Appressed	++	Rightangle
Mp-SIKR	Black	Greyishblack	Fluffy	+++	Rightangle
Mp-SNGR	Black	Greyishblack	Partiallyfluffy	++	Rightangle
Mp-UDZ	Black	Greyishwhite	Partiallyfluffy	+	Rightangle

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Indices:+= poor; ++=average;+++ =high;

Mycelialdry weight

The results depicted in Table 2showed significantdifferences among thecollected twelve isolates on the mycelia dry weight basis. MP-JSM produced a maximummycelial dry weight of 66.50 mg, and the least of mycelial dry weight was MP-JU (37.00mg). Out of twelve isolates, five isolates *viz*., Mp-BKN (60.20 mg), Mp-CUR (58.55 mg),Mp-DPA (63.50 mg), MP-JSM (66.50 mg) and Mp-UDZ (55.80 mg) were grouped in thehigh range of mycelial dry weight (>55 mg), four isolates, Mp-AWR (52.50 mg), Mp-JJN(53.10 mg), Mp-NGO (52.30 mg) and Mp-SIKR (44.25 mg) were in the range of (40-55 mg)and ranked as medium mycelia dry weight. In comparison, three isolates: Mp-HMH, Mp-JUand Mp-SNGR, weighted 37.45 mg, 37.00 mg and 38.55 mg, respectively and were ranked inthe lowrange(< 40mg)mycelia dryweight.

Table2Variationinradialgrowthandmyceliadryweightofdifferent isolatesof*M.phaseolina*

Isolates	Radial growth(mm)* after				Growthrate (mm/h)**	Mycelialdryweight (mg)
	24h	48h	72h	96h		
Mp-AWR	18.20	39.70	60.10	79.10	0.90	52.50
Mp-BKN	31.20	61.20	90.00	90.00	1.25	60.20
Mp-CUR	28.50	56.70	90.00	90.00	1.17	58.55
Mp-DPA	26.50	52.50	90.00	90.00	1.08	63.50
Mp-HMH	20.20	40.80	61.20	80.90	0.86	37.45
Mp-JJN	22.20	47.90	71.20	90.00	1.07	53.10
Mp-JSM	28.20	51.20	73.70	90.00	0.95	66.50
Mp-JU	25.70	49.30	74.50	90.00	0.98	37.00
Mp-NGO	22.50	47.90	71.90	90.00	1.06	52.30
Mp-SIKR	24.50	48.50	73.10	90.00	1.00	44.25
Mp-SNGR	21.50	41.90	62.70	82.90	0.85	38.55
Mp-UDZ	16.50	34.50	57.20	77.20	0.75	55.80
			SEm±			**1.29**
			CD5%			**3.77**

*Meanoffourreplications;**Growthrate(mm/h)measuredat 48 hours

Sclerotialcharacter

On the basis of microscopic observations significant variations wereobserved among these isolates regarding to the shape and size of the sclerotia. Results ofmicroscopic observation presented in Table 3and Plate 2 revealed that black to dark brown incolour, round to irregular in shape and significant variations were also observed among theseisolatesregardingthe numberandsizeoftheirsclerotia

All the isolates of *M. phaseolina*produced black colouredsclerotia except Mp-JSMandMp-UDZisolateswhichwereproducedbrowncolorsclerotia.Ontheshapebasissclerotia were categorized in three groups that were round, ovoid and irregular shape. Roundshaped sclerotia were recorded in Mp-BKN, Mp-CUR, Mp-DPA, Mp- JSM and Mp-SIKRisolate whereas ovoid-shaped sclerotia were recorded in Mp-AWR, Mp-HMH and Mp-JJNand in

Mp-JU, Mp-NGO, Mp-SNGR, and Mp-UDZ isolates sclerotia were irregular in shape.All the *M. phaseolina*isolates variedin their ability toproduce sclerotia on PDA medium,and based on the sclerotial number, the degree of sclerotial production were categorized asfew (1-20), several (21-40) and abundant (above 40). The maximum sclerotial number of 48per microscopic field was observed in Mp-BKN isolated, and the minimum sclerotial numberof15was observedintheMp-UDZisolate.

Abundant sclerotia formation was recorded in isolates Mp-BKN, Mp-CUR Mp-DPA,Mp-JSMandMp-JU(above40sclerotiaper10Xmicroscopicfield),several sclerotialproductions in Mp- HMH, Mp-JJN, Mp- NGO, Mp- SIKR and Mp-SGNR (21 to 39 sclerotiaper 10 X microscopic field) and deficient in remaining two isolates Mp-AWR and Mp- UDZ(1 to 20 sclerotia per 10X microscopic field). Significant variations regarding the size of theirsclerotiarangingbetween53.4-161.45µmwereobservedamongtheseisolates.ThemaximumsclerotialsizewasrecordedinMp-JSM(161.45µm)followedbyMp-DPA(148.75µm),Mp-HMH(127.55µm),Mp-AWR (114.2µm),Mp-BKN(101.2µm),Mp-CUR (98.85µm),Mp-SIKR(87.2µm),Mp-JU(84.45µm),Mp-SNGR(79.85µm),Mp-NGO (76.65 µm), Mp-JJN (65.2 µm) and minimum size sclerotia was observed in Mp-UDZ isolatewhich was 53.4 µm.Based on the size,sclerotia were classifiedinto three groups: large(>150 µm), medium (75-150 µm), small (<75 µm). Isolate Mp-JSM produced the largest sizeof sclerotia and categorized in the first large group. Medium size sclerotia were recorded innine isolates, *i.e*. Mp-DPA (148.75 µm), Mp-HMH (127.55 µm), Mp-AWR (114.2 µm), Mp-BKN(101.2µm),Mp-CUR(98.85µm),Mp-SIKR(87.2µm),Mp-JU(84.45µm),Mp-SNGR (79.85 µm), Mp-NGO (76.65 µm) and the smallest size sclerotia was found in Mp-UDZ(53.4µm).

Plate 2:Abundanceofsclerotiaindifferent isolatesof*M.* ***phaseolina***

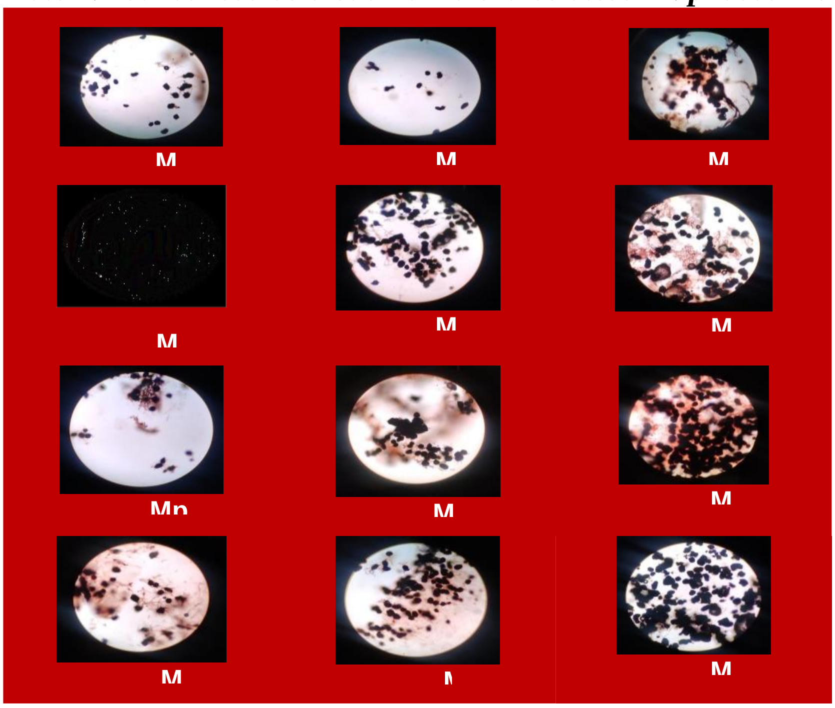

Table3Variationinabundance, colour, numbers, shapeandsizeofsclerotia

S. No.	Isolates	Abundance	Colour	Shape	Length (µm)	Width (µm)	Sclerotiasize (µm)*	No.ofsclerotia/ microscopic field(10x)**
1	Mp-AWR	+	Black	Ovoid	110.2	118.2	114.2	19
2	Mp-BKN	+++	Black	Round	105.3	98.4	101.85	48
3	Mp-CUR	+++	Black	Round	101.5	96.2	98.85	45
4	Mp-DPA	+++	Black	Round	152.4	145.1	148.75	43
5	Mp-HMH	++	Black	Ovoid	131.5	123.6	127.55	21
6	Mp-JJN	++	Black	Ovoid	68.3	62.1	65.2	33
7	Mp-JSM	+++	Brown	Round	171.2	151.7	161.45	44
8	Mp-JU	+++	Black	Irregularshape	86.7	82.2	84.45	41
9	Mp-NGO	++	Black	Irregularshape	81.2	72.1	76.65	36
10	Mp-SIKR	++	Black	Round	89.2	85.2	87.2	38
11	Mp-SNGR	++	Black	Irregularshape	82.5	77.2	79.85	24
12	Mp-UDZ	+	Brown	Irregularshape	61.4	55.4	53.4	15

**Meanof 20sclerotia
Indices+=Few,++=Several+++ =Abundant
*Sclerotiasizewascalculatedby(length+width)/2(mm)on5days

Effectofdifferentculture media

Variousfungi requiredifferentconstituentsinvaryingquantitiesfor theirgrowth and development; hence a particular medium may be suitable for a specific fungus.Keeping this in view, an experiment was conducted to find out a suitable culture medium forthe growth and sclerotial production of *M. phaseolina*, the causal pathogen of dry root rotdisease of clusterbean. Therefore, five solid media of various origin and composition wereused for this purpose: Potato Dextrose Agar, Czapek-Dox, Richard's and Host leaf extractagarand Oatmeal agarmedium.Results of the effect of differentculture media on themycelial growth of *M.phaseolina*isolates tabulatedin Table4.Basedon observation ofmean mycelia growth,for all the *M. phaseolina*isolates, the most superior culture media wasfound *viz.*, Potato Dextrose Agar (85.20 mm), Richard's agar (79.10 mm), Czapek'sdox(74.44 mm), oatmeal agar(69.05 mm) and Host leaf extract agar (62.80 mm). Mp-AWR(81.05mm),Mp-BKN(90.0mm),Mp-CUR(90.0mm),Mp-DPA(90.00mm),Mp-HMH (85.25mm),Mp-JJN(83.15mm)Mp-JSM(90.00mm),Mp-JU(88.15mm),Mp-NGO (86.20mm) Mp-SIKR (84.15mm), Mp-SGNR (81.30 mm) and Mp-UDZ (73.15 mm) attainedmaximum mycelial growth on Potato Dextrose Agar (PDA) as compared to Czapek'sdox,Richard'sagar,Hostleafextractagarandoatmealagarmedium.

Table4: Effectofdifferentculturemediaonmycelialgrowthof*Macrophominaphaseolina*isolates

S.No.	Isolates	Mycelialgrowth(mm)ondifferentculturemedia					Mean
		Czapek's(Dox)	PotatoDextrose Agar	Richard'sAgar	HostLeaf Extract Agar	Oat MealAgar	
1	Mp-AWR	71.22	81.05	80.25	63.20	68.20	72.78
2	Mp-BKN	81.25	90.00	83.50	71.23	80.90	81.37
3	Mp-CUR	87.21	90.00	83.85	73.21	81.20	83.09
4	Mp-DPA	77.20	90.00	81.23	61.25	60.25	73.98
5	Mp-HMH	83.00	85.25	83.10	61.26	71.00	76.72
6	Mp-JJN	60.00	83.15	78.23	58.90	61.95	68.44
7	Mp-JSM	81.00	90.00	82.55	64.32	76.50	78.87
8	Mp-JU	75.10	88.15	79.25	61.12	68.00	74.32
9	Mp-NGO	74.00	86.20	73.89	58.95	66.25	71.85
10	Mp-SIKR	73.12	84.15	78.90	60.25	69.00	73.08
11	Mp-SNGR	74.22	81.30	73.22	60.00	67.50	71.24
12	Mp-UDZ	56.00	73.15	71.23	59.90	57.95	63.64
	Mean	**74.44**	**85.20**	**79.1**	**62.80**	**69.05**	
				SEm±	**CD(P=0.05)**	**CV(%)**	
	Isolates			0.68	1.98	5.10	
	Media			0.53	1.54		
	IsolatesX Media			1.92	5.61		

On the basis of observationof mean mycelia growth,for all the *M. phaseolina*isolates the most superior culture media was found in order to Potato Dextrose Agar (85.20mm), Richard's agar (79.10 mm), Czapek'sdox (74.44 mm), Oat meal agar (69.05 mm) andHostleafextractagar(62.80mm).

Pathogenicvariability

A pot experiment was conducted to study the pathogenic variability of twelve *M.phaseolina*against five clusterbean varieties. The dry root rot incidence recorded in thesevarietiesin responsetotwelve*M.phaseolina*isolatesisgivenin Table5.Theresultsrevealed that *M. phaseolina*isolate Mp-BKN was highly virulent on all tested clusterbeanvarieties and maximum mean disease incidence (69.95%) was also recorded in this isolate.Highest disease incidence (75.35%) was recorded in variety RGC-986 with Mp-BKN isolatefollowed by varieties RGC-936 (71.65%), RGC-1038 (70.05%), RGC-1055 (69.25%) andRGC-1017(63.48%).NexthighervirulentisolatewasMp-CURonallthetestedfivevarieties RGC-986, RGC-936, RGC-1038, RGC-1055 and RGC-1017 with per cent diseaseincidence of (71.66%), (66.35%), (64.10%), (63.35%) and (61.23%), respectively. Mp-UDZwas least virulent on all the five varieties RGC-986, RGC-936, RGC-1038, RGC-1055 andRGC-1017 with per cent disease incidence of (19.09%), (37.45%), (36.24%), (34.95%) and(30.33%), respectively and minimum mean disease incidence (31.61%) was recorded withthisisolateinallthetestedfivevarieties.

Table5PathogenicvariabilityofMacrophominaphaseolinaisolatesagainstfiveclusterbeanvarieties

S.NO	Isolates	PercentdiseaseIncidence					MEAN
		Clusterbeanvarieties					
		RGC-986	RGC-936	RGC-1017	RGC-1055	RGC-1038	
1	Mp-AWR	32.47 (34.74)	46.29 (42.87)	36.56 (37.20)	40.23 (39.37)	42.33 (40.59)	39.58 (38.98)
2	Mp-BKN	75.35 (60.23)	71.65 (57.83)	63.48 (52.82)	69.25 (56.32)	70.05 (56.82)	69.95 (56.76)
3	Mp-CUR	71.76 (57.90)	66.35 (54.54)	61.23 (51.49)	63.35 (52.74)	64.10 (53.19)	67.96 (55.52)
4	Mp-DPA	63.83 (59.23)	59.65 (50.56)	56.50 (48.73)	57.95 (49.57)	58.37 (49.82)	61.26 (51.51)
5	Mp-HMH	25.34 (30.22)	41.75 (40.25)	32.90 (35.00)	37.45 (37.73)	39.45 (38.91)	35.38 (36.50)
6	Mp-JJN	39.45 (38.91)	48.26 (44.00)	40.26 (39.38)	43.33 (41.17)	45.95 (42.68)	43.45 (41.24)
7	Mp-JSM	66.05 (60.70)	62.93 (52.49)	59.20 (50.30)	61.45 (51.62)	63.05 (52.56)	64.54 (53.45)
8	Mp-JU	54.23 (47.43)	56.20 (48.56)	52.56 (46.47)	54.98 (47.86)	56.50 (48.73)	54.89 (47.81)
9	Mp-NGO	42.75 (40.83)	51.45 (45.83)	45.55 (42.45)	47.90 (43.80)	49.10 (44.48)	47.35 (43.48)
10	Mp-SIKR	48.55 (44.17)	54.30 (47.47)	49.92 (44.95)	51.20 (45.69)	52.55 (46.46)	51.30 (45.75)
11	Mp-SNGR	27.50 (31.63)	43.25 (41.12)	35.95 (36.84)	39.92 (39.18)	41.20 (39.93)	37.56 (37.80)
12	Mp-UDZ	19.09 (25.91)	37.45 (37.73)	30.33 (33.42)	34.95 (36.24)	36.24 (37.01)	31.61 (34.21)
	MEAN	51.53 45.88	53.29 46.89	47.04 43.30	50.16 45.09	51.57 45.90	

	SEm±	CD5%
Isolates	1.38	4.20
Genotypes	1.26	3.83
IsolateX Genotype	1.87	5.62

*Figuresinparenthesesareangulartransformedvalues

Conclusion

Twelve *M. phaseolina*isolates collected during the survey were studied for their cultural,morphological and pathological variability. On the radial growth basis, three isolates Mp-DPA,Mp-BKN, and MpCUR, are considered fast-growing, five isolates viz., Mp-JU, Mp-JSM Mp-JJN Mp-NGO and Mp-SIKR were categorized as medium growing while four isolates viz., Mp-AWR, Mp-HMH, Mp-SNGR, Mp-UDZ considered as slow-growing. The basis on the visualobservations colony colour of *M. phaseolina*appeared as black Mp-BKN (dark black), Mp-AWR(Black),Mp-CUR(Black),Mp-DPA(greyishblack),Mp-HMH(greyishblack),Mp-JSM(black), Mp-SIKR (greyish black) and Mp-SGNR (Greyish black), Mp-JJN (greyish white) andMp-NGO(creamy white),Mp-UDZ(greyish-white)andMp-JU(lightbrown).Highaerialmycelium growth (+++), was recorded in Mp-BKN, Mp-DPA, MpJJN and Mp- SIKR isolatesand poor aerial mycelium growth (+) in MpAWR and Mp-UDZ isolates, and all the isolates hadright-anglebranchingpattern.

On the basis of colony texture, four isolates *viz*., MpBKN, Mp-HMH, Mp-JSM and Mp-NGO produced appressed colony while three isolates *viz*., Mp-DPA, Mp-Mp-JJN and Mp-SIKRhad fluffy texture colony and remaining five isolates Mp-AWR, Mp-Mp-CUR, MpJU, Mp-SNGR and Mp-UDZ produced partially fluffy colony. Most isolates produced black colouredsclerotia except Mp-JSM and MpUDZ isolates, which were brown in colour. On a shape basis,three types of sclerotia were observed: round, ovoid and irregular, ranging between 53.4-161.45µm and 15-48 per microscopic field, respectively. The maximum sclerotial number of 48 permicroscopic field was observed in Mp-BKN isolated, and the minimum sclerotial number of 15wasobservedintheMp-UDZisolate.

Mp-JSM (161.45 µm) produced maximum sclerotial size, and Mp-UDZ isolate producedminimum sclerotia (53.4 µm). All the five tested solid media of various origin and compositionsupportedthemycelialgrowthof*M.phaseolina*isolates.Amongthetestedmedia,PotatoDextrose Agar (PDA) found best. All the isolates attained maximum mycelial growth with meanmycelia growth of 85.20 mm and minimum mycelial growth of all the isolates observed on HostLeaf ExtractAgar. All the *M. phaseolina*isolates tested for their pathogenic variability, usingfiveclusterbeanvarieties.Mp-BKNalsofoundhighlypathogeniconalltestedfivevarietieswith 69.95 per cent mean disease incidence and maximum disease incidence (75.35%) was recordedon culsterbean variety RGC-986 and minimum (63.48%) on variety RGC-1017. Mp-UDZ isolatewasleastvirulentonallthetestedfivevarietieswithminimummeandiseaseincidence(31.61%).

Practically there was no direct correlation found between morphological and culturalcharacters and virulence except the abundance of sclerotia production in any of the isolates. Fivehighly virulent isolates *viz*., Mp-BKN, Mp-CUR, Mp-DPA, Mp-JSM, Mp-JU produced abundantsclerotia. Noapparentcorrelation was observedbetween growth rate, colony color, texture;mycelia dry weight, shape, size of sclerotia and the virulence. The present finding concluded thatthedegreeofsclerotiaproductionispositivelycorrelatedwiththevirulenceasreflectedbythe abovehighlypathogenicisolates.Thesepresentinvestigationresultsagreewith Shekha*etal*.(2006), Tanaji*etal*.(2017)andManjunathaandSaifulla(2018).

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