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Main Oilseed Research Station, Junagadh Agricultural University, Junagadh, Gujarat, India Physiological variability of *Macrophomina* phaseolina (Tassi) Goid. Isolates causing root rot of castor

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Abstract

The fungus *Macrophomina phaseolina* is the devastating fungus of many crops. For this study, infected maize samples were collected from castor growing areas of Gujarat. Twenty-five isolates of *M. phaseolina* were isolated from infected castor samples and their growth was evaluated at 20, 25, 30, 35 and 40°C as well as at 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 pH. The growth of fungal isolates was significantly affected by different levels of temperature and pH. Among different five temperature levels, significantly maximum mean growth (89.53 mm) was observed at 35 °C temperature followed by 30 °C(82.46 mm) after four days of incubation on PDA medium. Among different seven pH levels, significantly maximum mean growth of dry mycelial (352.42 mg) was recorded at pH 7.0 followed by pH 6.5 (313.70 mg) and pH 6.0 (311.02 mg), however pH 6.5 and pH 6.0 were statistically at par with each other. Among different seven pH levels, significantly maximum mean growth of dry mycelial (352.42 mg) was recorded at pH 7.0 followed by pH 6.5 (313.70 mg) and pH 6.0 (311.02 mg), however pH 6.5 (313.70 mg) and pH 6.0 (311.02 mg), clearly indicated the preference of isolates to particular range of pH. Mean dry mycelial weight was increased with increase in pH and temperature.

Keywords: Macrophomina phaseolina, physiological variability, Ricinus communis L.

Introduction

Castor (Ricinus communis L.) is mainly grown in tropical, subtropical as well as temperate regions covering about 30 different countries on commercial scale. Among these India is the world's principal producer of castor and ranks first both in area and production. Major castor growing states in India, Gujarat ranks first in area and production, contributing about 71.00 per cent area and 80 per cent of the country production (Anon., 18)^[1]. Castor crop is affected by several biotic and abiotic stresses, 15 different diseases have been recorded on castor crop in India (Kolte, 1995)^[4], which cause serious quantitative and qualitative losses at different crop growth stages depending upon seasonal condition s. The fungus Macrophomina phaseolina being the most serious castor bean pathogen in dry and warm conditions. This disease appears at different growth stages of crops and hence, it is named differently as spike blight, stem blight, twig blight, collar rot and root rot (Moses and Reddy, 1987)^[7]. The fungus, M. phaseolina is very infective during hot and dry season and cause heavy seed yield losses. Generally, in rainfed castor cultivation, root rot is the major problem. The fungus M. phaseolina is soil and seed-borne pathogen whichproduces cushion shaped black sclerotia and was found associated with more than 500 crop and non-crop species (Smith and Carvil, 1977) ^[13].Not much work has been done on the variability among the isolates of *M. phaseolina* caused castor root rot and needs special attention. Since breeding for disease resistance is based on the knowledge of existence of variation in the pathogen population. Hence, the present investigation, information on physiological variability among 25 isolates of M. phaseolina causing castor root rot is reported.

Materials and Methods

Collection of fungal isolates

A total of 25 diseased samples were collected from castor growing areas in Gujarat (Table 1). Samples of typically infected root and stem portion near the collar region characteristic symptoms of root rot were collected from the farmers' fields. The diseased samples were first

Corresponding Author: Pinal Vekariya Main oilseed Research Station, Junagadh Agricultural University, Junagadh, Gujarat, India packed in paper bags (20×15 cm) and then properly labelled, brought to the laboratory and stored at room temperature for further study.

Isolation and storage of M. phaseolina

Isolation of the pathogen was made from each diseased specimen separately by tissue isolation technique. The purified culture (5mm disc) of each of the isolates growing on PDA containing culture tubes and incubated in the dark at 30 \pm 2°C temperature for four days, until the slant of each of the isolate was covered with a dense sclerotial layer of the fungal culture. The culture tubes were labelled and stored at 4°C temperature for further study.

Temperature

To study the effect of temperature on growth of 25 isolates of M. phaseolina, by growing the isolates separately at five temperature levels viz., 20, 25, 30, 35 and 40°C on PDA medium. For study on different temperature, PDA medium was sterilized through autoclaving at 1.045 kg/cm²pressure for 20 minutes. The pH of all the media was adjusted to 6.0 by using 0.1N hydrochloric acid (HCl) or 0.1 N sodium hydroxide (NaOH) solutions. Medium (20 ml) was poured in Petri dishes under aseptic conditions and leave for solid. Four days old culture discs (5 mm) of each isolate were inoculated separately and incubated at different temperature levels. Each isolate was replicated two times. After four days of incubation growth rate of each of the isolates in different temperature were measured in term of colony diameter and their means were computed. On the basis of radial growth, the isolates were categorized in groups as slow (≤ 65 mm), medium (> 65-80 mm) and fast (> 80 mm).

Hydrogen-ion concentration

The 25 isolates of *M. phaseolina* were grown separately on potato dextrose broth in selected pH levels of 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. For study on different pH level, potato dextrose broth was sterilized through autoclaving at 1.045 kg/cm² pressure for 20 minutes. The pH levels were adjusted by using 0.1 N HCl or 0.1 N NaOH solutions with the help of pH meter. Four days old culture discs (5 mm) of each isolates were inoculated separately into conical flasks containing 50 ml of medium at different pH levels. After 15 days of incubation at 30 \pm 2 °C, the mycelial growth was harvested, on previously weighted, oven dried whatsman's filter paper No 41 giving sufficient washing with warm distilled water. The filter papers with mycelial mats were dried in an oven at 60°C temperature till constant weight was obtained and each isolates categorized as different group on the basis of dry mycelial growth as less (≤ 300 mg), medium (> 300-500 mg) and high (> 500 mg).

Result and Discussion

Physiological variability of different isolates of castor root rot pathogen

Response of temperature

Variation in respect of mean growth was observed among different temperature levels as well as isolates.

Among different five temperature levels, significantly maximum mean growth (89.53mm) was observed at 35 °C temperature followed by 30 °C(82.46mm) after four days of incubation on PDA medium, while minimum mean growth (51.01mm) was observed at 40 °C temperature followed by 20 °C (57.80 mm).

Among different 25 isolates, significantly maximum mean growth (80.10 mm) was produced by Mp-13 followed by Mp-18 (78.35 mm). Better mean growth (76.90 mm) was also produced by Mp-1 followed by Mp-7 (76.65 mm) and were statistically at par. Minimum mean growth (53.70 mm) was produced by Mp-3 followed by Mp-6 (62.45 mm), Mp-5 (62.85 mm) Mp-10 (63.80 mm)and Mp-2 (63.95 mm), however isolates Mp-2, Mp-5, Mp-6 and Mp-10 were statistically at par with each other.

Interaction of different isolates and temperature levels also showed significant variation in respect of growth. Most of the isolates supported maximum growth at 35 °C temperature except Mp-9, 11, 15, 17 and 20 which supported maximum growth at 30 °C temperature as compared to other temperature levels tested (Table 2).

Based on growth, 25 isolates were categorized into three groups, *viz.*, slow, medium and fast. Isolates Mp-2, 3, 11 and 17 grew slow at 20 °C, 25 °C, and 40 °C temperature, but fast at 30 °C and 35 °C temperature. The isolate Mp-6 grew slow at 20 °C, 25 °C and 40 °C temperature, but grew medium at 30 °C and fast at 35 °C temperature. Similarly isolate Mp-22 grew slow at 25 °C, medium at 20 °C and 40 °C temperature, but fast at 30 °C and 35 °C temperature. The isolate Mp-4 grew slow at 20 °C and 40 °C temperature. The isolate Mp-4 grew slow at 20 °C and 40 °C temperature. The isolate Mp-4 grew slow at 20 °C and 40 °C temperature. The isolate Mp-4 grew slow at 35 °C temperature (Table 3).

Temperature is the most important physical factor influencing the growth of *M. phaseolina* depending on host crops and isolates collected from which climatic area. Maniciet al., (1995)^[6] reported the variability of *M. phaseolina* isolates of sunflower, collected from different climatic area considerably at different temperature levels. Rodrigues et al., (1997)^[9] reported that the highest production of pycnidia of M. phaseolina occurred on soybean agar with filter paper at 35°C. Devi and Singh (1998)^[3] noticed that all isolates of M. phaseolina grew best in Richard's medium and produced maximum sclerotia at 30 to 35°C temperature. Sharma et al. (2004)^[11] also observed that a higher temperature range of 25 to 35 °C favoured the growth of all four isolates of M. phaseolina isolated from pearl millet, sesame, horse gram and moth bean. Csondes *et al.* (2007)^[2] observed most favourable temperature regime was 25 to 35°C for all the 25 isolates of *M. phaseolina*. Sukanya *et al.*, (2016)^[14] reported the isolates of charcoal rot pathogen preferred 30°C temperature for their growth. There was a significant difference among the isolates (*M. phaseolina*), temperature levels (20 to 40° C) and their interactions (Kumar and Guar, 2017; Waqas et al., 2017)^[5, 16]. The results of present findings are in conformity with the above research workers. The 25 isolates of castor root rot pathogen varied at different temperature levels and their interactions significantly in their relatives growth. Most of the isolates preferred 35 °C temperature followed by 30 °C.

Response of Hydrogen-ion concentration

To study the variability among 25 isolates of *M. phaseolina*, seven pH levels from 5.0 to 8.0 with the range of 0.5 unit in each case were tested in potato dextrose broth medium. In this study, growth of dry mycelium of 25 isolates at seven pH levels was recorded. Variation in respect of growth of dry mycelium was observed among different pH levels as well isolates.

Among different seven pH levels, significantly maximum mean dry mycelial (352.42 mg)was at pH 7.0 followed by pH 6.5 (313.70 mg) and pH 6.0 (311.02 mg), however pH 6.5 and pH 6.0 were statistically at par with each other. Minimum

mean dry mycelial growth (207.42 mg) was recorded at pH 8.0 followed by pH 5.0 (247.24 mg).

Among different 25 isolates, significantly maximum mean dry mycelial growth (398.86 mg) was produced by Mp-14 followed by Mp-2 (364.00 mg) and Mp-4 (353.57 mg). Better mean dry mycelial growth was also produced in Mp-21 (335.00 mg) followed by Mp-23 (326.00 mg). Minimum mean dry mycelial growth (221.29 mg) was produced by Mp-24 followed by Mp-19 (228.86 mg).

Interaction of different isolates and pH levels also showed significant variation in respect of dry mycelial growth. The isolates Mp-8, 11 and 21 produced maximum dry mycelial growth at pH 5.5, while the isolates Mp-6 and Mp-19 produced maximum at pH 6.0, the isolates Mp-9, 12, 14, 22 and 23 produced maximum at pH 6.5, isolates Mp-1, 3, 4, 5, 7, 10, 13, 15, 17, 18 and 25 produce maximum at pH 7.0 and isolates Mp-16 and Mp-24 produced maximum at pH 7.5 than other pH levels tested (Table 4).

Based on dry mycelium growth, 25 isolates were categorized into three groups such as less, medium and high. The isolates Mp-12, 22 and 24 produced less dry mycelial growth and the isolate Mp-2 produced medium in all the seven pH levels tested. The isolates Mp-17 and Mp-18 produced less dry mycelial growth at pH 5.0, 5.5, 6.0, 7.5 and 8.0, but produced medium growth at pH 6.5 and 7.0. The isolates Mp-16 produced less dry mycelial growth at pH 5.0, 5.5, 6.0, 5.5, 6.0, 6.5, 7.0 and 8.0 but medium at pH 7.5, while isolate Mp-19 produced less dry mycelial growth at pH 5.0, 5.5 6.5, 7.0, 7.5 and 8.0, but

medium at pH 6.0. The isolates Mp-3 and Mp-14 produced medium dry mycelial growth at pH 5.0, 5.5, 6.0, 7.0, 7.5, 8.0, but at pH 6.5 Mp-3 produced less and Mp-14 produced higher dry mycelial growth. The isolates Mp-10, 15 and 20 produced less dry mycelial growth at pH 5.0, 5.5, 6.0, 6.5, 7.5 and 8.0, but produced medium at pH 7.0 (Table 5).

The hydrogen-ion concentration is one of the most important factors influencing growth of M. phaseolina at different pH levels. Ratnoo and Bhatnagar (1991)^[8] reported that the growth increased gradually with increase in pH level up to 7.0 and thereafter, it declined. Sahi et al. (1992) [10] observed optimum growth of *M. phaseolina* at pH 6.5, but also good at pH 6.0. Singh and Kaiser (1994)^[12] also reported optimum growth of M. phaseolina at pH 6.5 and good at pH 5, pH 6 and pH 7.0. Sharma *et al.* (2004) ^[11] observed that pH between 6.5 to 7.0 favoured the growth of all four isolates of M. phaseolina from different crops. Sundravadana and Thirumurugan (2012) ^[15] observed that the pH 7.0 had supported the mycelial growth of *R*. *bataticola* isolates from roots, leaf and seeds of pulses and there was drastic reduction of mycelial growth at pH 8.0. Waqas et al. (2017) [16] concluded that higher mean dry mycelial weight of M. phaseolina from maize was observed at pH 6.5 and 7.0. The results of present findings are in conformity with the above research workers. The 25 isolates of castor root rot pathogen greatly varied against seven pH levels in respect of dry mycelia growth.

Table 1: List of isolates of M.	nhaseoling obtained from	different locations of	ofeastor growing ar	eas of Guiarat
TADIC I. LIST OF ISOTATES OF M.	phaseouna obtained nom	unificient locations (Jicastor growing are	zas or Oujarai

Sr. No.	Isolates	Location						
Sr. No.	Isolates	Village	Taluka	District				
1	Mp-1	Sardarkrushinagar	Dantiwada	Banaskantha				
2	Mp-2	Silasana	Dhanera	Banaskantha				
3	Mp-3	Gathaman	Palanpur	Banaskantha				
4	Mp-4	Pepalu	Lakhani	Banaskantha				
5	Mp-5	Bhakhar	Dantiwada	Banaskantha				
6	Mp-6	Jaska	Vadnagar	Mahesana				
7	Mp-7	Vadali	Vadali	Sabarkantha				
8	Mp-8	Thalvada	Vadnagar	Mahesana				
9	Mp-9	Karnasar	Tharad	Banaskantha				
10	Mp-10	Gangundra	Dantiwada	Banaskantha				
11	Mp-11	Laxmipura	Unjha	Mahesana				
12	Mp-12	Santalpur	Vanthali	Junagadh				
13	Mp-13	Bagadu	Junagadh	Junagadh				
14	Mp-14	Araniyala	Mendarda	Junagadh				
15	Mp-15	Dervan	Keshod	Junagadh				
16	Mp-16	Lushala	Vanthali	Junagadh				
17	Mp-17	Barawala	Mendarda	Junagadh				
18	Mp-18	Mendarda	Mendarda	Junagadh				
19	Mp-19	Khumbhadi	Vanthali	Junagadh				
20	Mp-20	Khorasha	Vanthali	Junagadh				
21	Mp-21	Sogadi	Jamjodhpur	Jamnagar				
22	Mp-22	Khadpipali	Mendarda	Junagadh				
23	Mp-23	Dhutarpar	Jamjodhpur	Jamnagar				
24	Mp-24	Jamnagar	Jamnagar	Jamnagar				
25	Mp-25	Supedi	Dhoraji	Rajkot				

Table 2: Growth of twenty five isolates of *M. phaseolina* at five different temperature levels

Sr. No.	Isolates		Mean				
		20	25	30	35	40	
1	Mp-1	65.00	82.00	87.25	90.00	60.25	76.90
2	Mp-2	50.00	58.00	86.25	90.00	35.50	63.95
3	Mp-3	24.75	45.00	80.50	89.50	28.75	53.70
4	Mp-4	60.50	75.75	79.25	89.25	43.25	69.60

5	Mp-5	54.25	70.75	79.00	89.75	20.50	62.85	
6	Mp-6	50.25	64.00		89.75	42.75	62.45	
7	Mp-0 Mp-7	63.75	85.50		89.25	57.25	76.65	
8	Mp-8	52.85	59.50		90.00	64.50	67.02	
9	Mp-9	57.50	86.50		89.00	48.50	74.20	
10	Mp-10	49.75	55.25		88.25	46.75	63.80	
11	Mp-11	55.75	61.25	89.25	88.75	49.25	68.85	
12	Mp-12	63.00	77.00		90.00	55.50	72.80	
13	Mp-13	75.50	75.00	85.50	90.00	74.50	80.10	
14	Mp-14	56.75	66.50	82.50	90.00	48.75	68.90	
15	Mp-15	43.25	53.75	89.25	88.00	66.00	68.05	
16	Mp-16	69.25	65.50	75.25	89.50	28.50	65.60	
17	Mp-17	57.25	59.25	90.00	88.75	51.50	69.35	
18	Mp-18	63.75	75.25	85.75	90.00	77.00	78.35	
19	Mp-19	48.75	53.25	78.50	89.50	74.25	68.85	
20	Mp-20	66.75	73.25	89.50	89.00	48.25	73.35	
21	Mp-21	57.00	66.50	87.75	90.00	50.75	70.40	
22	Mp-22	60.25	62.75	87.75	90.00	70.25	74.20	
23	Mp-23	60.75	69.75	80.75	90.00	47.00	69.65	
24	Mp-24	66.75	75.00	76.25	90.00	56.75	72.95	
25	Mp-25	71.75	81.25	83.00	90.00	29.00	71.00	
Mean		57.80	67.90	82.46	89.53	51.01		
		Temperature		Isolates		Temperature × Isolates		
S	. Em±	0.25		0.56		1.25		
C.I	D. at 5%	0.70		1.57		3.51		

*Mean of two replications

Table 3: Grouping of twenty five isolates of *M. phaseolina* based on growth (colony diameter) at five different temperature levels

6		Growth	Isolates								
Sr.	Crown				re (°C)						
No. Group			20	25	30	35	40				
1	Ι	Slow (≤65)	1, 2, 3,4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 17, 18, 19, 21, 23,	2, 3, 6, 8, 10, 11, 15, 17,19, 22	-		1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 17, 20, 21, 23, 24, 25				
2	Π	Medium (> 65-80)	13, 16, 20, 22, 24, 25	4, 5, 12, 13, 14, 16, 18, 20, 21, 23, 24	4, 5, 6, 8, 10, 12, 16, 19, 24		13, 15, 18, 19, 22				
3	III	Fast (> 80)		1, 7, 9, 25		13, 14, 15, 16, 17, 18, 19, 20, 21,					

Table 4: Growth of twenty five isolates of *M. phaseolina* at seven different pH levels

Sr.		Dry mycelial weight (mg)*								
No.	Isolates	рН								
140.		5.0	5.5	6.0	6.5	7.0	7.5	8.0		
1	Mp-1	272.50	280.50	287.00	335.00	503.50	315.00	177.50	310.14	
2	Mp-2	387.00	445.50	335.00	305.00	405.50	368.00	302.00	364.00	
3	Mp-3	300.50	305.50	328.50	238.50	341.00	300.50	302.00	302.36	
4	Mp-4	323.50	305.00	351.00	460.00	501.00	325.50	209.00	353.57	
5	Mp-5	262.00	315.00	271.50	285.00	505.00	337.50	158.50	304.93	
6	Mp-6	185.50	376.50	500.50	280.00	278.00	254.50	197.50	296.07	
7	Mp-7	225.00	237.00	250.50	298.50	408.00	308.00	184.50	273.07	
8	Mp-8	321.50	502.00	388.00	294.00	293.50	147.00	129.50	296.50	
9	Mp-9	128.00	238.50	298.00	500.50	329.00	321.50	300.50	302.29	
10	Mp-10	207.00	235.00	267.00	282.00	351.50	248.00	247.00	262.50	
11	Mp-11	356.50	363.00	231.50	200.00	198.00	180.00	176.50	243.64	
12	Mp-12	270.00	281.00	295.00	300.00	298.50	265.00	204.00	273.36	
13	Mp-13	168.00	227.50	278.00	312.00	402.50	245.00	201.00	262.00	
14	Mp-14	300.50	380.00	474.00	508.00	438.00	358.00	333.50	398.86	
15	Mp-15	206.50	270.00	284.50	300.00	389.00	214.50	168.50	261.86	
16	Mp-16	176.00	204.00	248.50	258.00	265.00	393.50	125.00	238.57	
17	Mp-17	156.50	200.00	238.50	305.50	384.00	300.50	132.50	245.29	
18	Mp-18	187.00	205.00	287.00	308.50	405.50	300.00	252.00	277.86	
19	Mp-19	245.50	260.00	334.00	242.50	201.00	174.00	145.00	228.86	
20	Mp-20	189.50	224.50	248.00	260.50	368.50	268.00	200.00	251.29	
21	Mp-21	350.00	487.00	412.00	305.00	298.00	268.00	225.00	335.00	
22	Mp-22	300.00	298.00	277.00	259.50	238.00	197.00	180.00	249.93	
23	Mp-23	293.00	338.00	347.00	500.50	321.50	298.50	183.50	326.00	
24	Mp-24	153.00	183.00	193.50	205.00	298.00	299.50	217.00	221.29	

25	Mp-25	216.50	288.00	350	0.00	299.00	389.00	304	.00	234.00	297.21	
	Mean	247.24	297.98	311	.02	313.70	352.42	279	.42	207.42		
			pН		Isolates				pH × Isolates			
	S. Em±	1.26				2.38			6.29			
C.	.D. at 5%	3.78			6.64			17.58				

*Mean of two replications

Table 5: Grouping of twenty five isolates of *M. phaseolina* based on growth of dry mycelium grown at seven different pH levels

6		Day myoslio				Isolates							
Sr.	Group	Dry mycelia weight (mg)		рН									
110.		weight (ing)	5.0	5.5	6.0	6.5	7.0	7.5	8.0				
1	Ι	Less(≤ 300)	1, 5, 6, 7, 9,1012, 13, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25		1, 5, 7, 9, 10, 11, 12, 13, 15, 16, 17, 18, 20, 22, 24		6, 8, 11, 12, 16, 19, 21, 22, 24	6, 8,10, 11, 12, 13, 15, 18,19, 20, 21, 22, 23, 24	1, 4, 5, 6, 7, 8, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25				
2	II	Medium (>300-500)	2, 3, 4, 8, 11, 14, 21		2, 3, 4, 8, 14, 19, 21, 23, 25	1, 2, 4, 13, 17, 18, 21	2, 3, 7, 9, 10, 13, 14, 15, 17, 18, 20, 23, 25	1, 2, 3, 4, 5, 7, 9, 14, 16, 17, 25	2, 3, 9, 14				
3	III	High(> 500)	-	8	6	9, 14, 23	1, 4, 5	-	-				

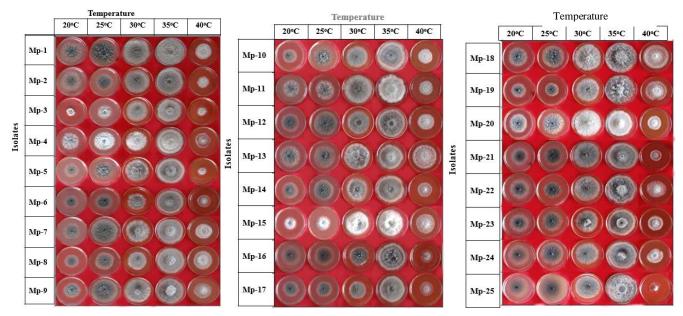


Fig 1: Growth of twenty five isolates of M. phaseolina at five different temperature levels

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