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GROWTH RATE, PATHOGENICITY AND FUNGICIDE SENSITIVITY OF *Macrophomina* spp. FROM WEEDS, MELON AND WATERMELON ROOTS[1]

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ABSTRACT - *Macrophomina* (Botryosphaeriaceae) is one of the main genera of soilborne phytopathogenic fungi, which causes root and seed rot in more than 800 host plants worldwide. Recent phylogenetic studies identified the species *M. phaseolina* and *M. pseudophaseolina* in *Trianthema portulacastrum* and *Boerhavia diffusa* in melon and watermelon production areas in northeastern Brazil. Therefore, the objective of this study was: i) to verify the effect of temperature and salinity on the mycelial growth of *M. phaseolina, M. pseudophaseolina* and *M. euphorbiicola*, ii) to assess their pathogenicity on melon and watermelon seedlings, and iii) to determine their sensitivity to the fungicide carbendazim. The optimal temperature for mycelial growth rate (MGR) for *Macrophomina* spp. ranged from 27.18 ºC (CMM4771 – *M. pseudophaseolina*) to 31.80 ºC (CMM4763 – *M. phaseolina*). For the effect of salinity on mycelial growth of *Macrophomina* isolates, the EC_{50} ranged from 103.76 (CMM4868 – *M. euphorbiicola*) to 315.25 mM (CMM4801 – *M. pseudophaseolina*). The pathogenicity test demonstrated that *M. phaseolina*, *M. pseudophaseolina* and *M. euphorbiicola* are pathogenic on melon with *M. phaseolina* exhibiting a higher level of virulence. *Macrophomina euphorbiicola* isolates did not cause disease in watermelon. The most sensitive isolates to the fungicide carbendazim were CMM4868, CMM4867 (*M. euphorbiicola*) and CMM1531 (*M. phaseolina*) with EC_{50} of 0.003, 0.012 and 0.012 mg.L^{-1} a.i., respectively. All *Macrophomina* spp. used in these experiments were pathogenic to the tested melon and watermelon cultivars with the exception of the *M. euphorbiicola* isolate that did not cause damage to watermelon.

Keywords: *Citrullus lanatus. Cucumis melo*. Salinity. Soilborne fungi. Temperature.

ESTUDO DO CRESCIMENTO MICELIAL DE *MACROPHOMINA* SPP. NO BRASIL, SUA PATOGENICIDADE E SENSIBILIDADE A FUNGICIDA

RESUMO - *Macrophomina* (Botryosphaeriaceae) é um dos principais gêneros de fungos fitopatogênicos de solo, que causam apodrecimento de raízes e sementes em mais de 800 plantas hospedeiras em todo o mundo. Estudos filogenéticos recentes identificaram as espécies *M. phaseolina* e *M. pseudophaseolina* em *Trianthema portulacastrum* e *Boerhavia diffusa* em áreas de produção de melão e melancia no Nordeste do Brasil. Portanto, o objetivo deste estudo foi: i) verificar o efeito da temperatura e salinidade sobre o crescimento micelial de *M. phaseolina*, *M. pseudophaseolina* e *M. euphorbiicola*, ii) avaliar sua patogenicidade em mudas de melão e melancia, e iii) determinar suas sensibilidades ao fungicida carbendazim. A temperatura ótima para taxa de crescimento micelial (MGR) para *Macrophomina* spp. variou de 27,1 ºC (CMM4771 – *M. pseudophaseolina*) a 31,8 ºC (CMM4763 – *M. phaseolina*). Para o efeito da salinidade no crescimento micelial de isolados de *Macrophomina*, a EC_{50} variou de 103,76 (CMM4868 – *M. euphorbiicola*) a 315,25 mM (CMM4801 – *M. pseudophaseolina*). O teste de patogenicidade demonstrou que *M. phaseolina*, *M. pseudophaseolina* e *M. euphorbiicola* são patogênicas em melão com *M. phaseolina* apresentando maior virulência. Isolados de *Macrophomina euphorbiicola* não causaram doenças em melancia. Os isolados mais sensíveis ao fungicida carbendazim foram CMM4868, CMM4867 (*M. euphorbiicola*) e CMM1531 (*M. phaseolina*) com EC_{50} de 0,003; 0,012 e 0,012 mg.L^{-1} i.a., respectivamente. Todas as espécies de *Macrophomina* spp. utilizados nestes experimentos foram patogênicos para as cultivares de melão e melancia testadas com exceção dos isolados de *M. euphorbiicola* que não causaram danos à melancia.

Palavras-chave: *Citrullus lanatus. Cucumis melo*. Salinidade. Fungos habitantes do solo.Temperatura.

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INTRODUCTION

Macrophomina phaseolina (Tassi) Goid. (GOIDÀNICH, 1947) (Botryosphaeriaceae, Ascomycota) is a *soil-borne pathogenic* fungus with a worldwide distribution on over 800 species of plant including economically important hosts such as soybean [*Glycine max* (L) Merrill], melon (*Cucumis melo* L.), watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], common bean (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* L. Walp), sorghum (*Sorghum bicolor* L.) and cotton (*Gossypium herbaceum* L.) (SALES JÚNIOR et al., 2020; LODHA; MAWAR, 2020; FARR; ROSSMAN, 2022). It causes different diseases: charcoal rot, grey stem rot, root rot, damping-off and seed damage, being more aggressive in tropical and subtropical countries, with a semiarid climate. In addition, the ability of this pathogen to survive in soil and/or crop residues through resistance/survival structures (microsclerotia), as well as in seeds, makes its management quite difficult (DHINGRA; SINCLAIR, 1978).

For a long time, it was considered that only one *Macrophomina* species (*M. phaseolina*) existed, however, phylogenetic studies, carried out in the last decade, have shown the existence of high genetic variability in the genus *Macrophomina* (SARR et al., 2014; MACHADO et al., 2019; ZHAO et al., 2019). Consequently, four new species of *Macrophomina* have been recently described: *M. pseudophaseolina* Crous, Sarr and Ndiaye found in crops of *Abelmoschus esculentus* (L.) Moench., *Arachis hypogaea* L., *Hibiscus sabdariffa* L. and *Vigna unguiculata* (L.) Walp. in Senegal (SARR et al., 2014), *A. hypogaea*, *Gossypium hirsutum* L., *Ricinus communis* L., *Jatropha curcas* L. (MACHADO et al., 2019) and *Manihot esculenta* C. (BRITO et al., 2019), and in weeds of *Boerhavia diffusa* L. and *Trianthema portulacastrum* L. in Brazil (NEGREIROS et al., 2019); *M. euphorbiicola* A.R. Machado, D.J. Soares and O.L. Pereira in *R. communis* and *Jatropha gossypifolia* L. in Brazil (MACHADO et al., 2019); *M. vaccinii* Y. Zhang ter and L. Zhao in *Vaccinium* spp. in China (ZHAO et al., 2019); and more recently, *M. tecta* Vaghefi, B. Poudel & R.G. Shivas in *S. bicolor* in Australia (POUDEL et al., 2021). The study of adaptability components, widely used for new species of fungi, such as sensitivity to salinity and fungicide, mycelial growth at different temperatures and virulence has been very useful for evaluating the variability of the adaptability of isolates in populations of plant pathogenic fungi. However, as adaptability has a relative character, it must be estimated by measuring characters that reduce some adaptive advantage among individuals (CORREIA et al., 2014). In addition, the competitive capacity among populations of fungi can be inferred indirectly through these adaptability components (ZHAN; McDONALD, 2013). Thus, data on pathogenicity and adaptability of isolates can directly influence the management measures to be adopted in field production (MENGISTU et al., 2018).

So far, studies on adaptability components comparing the different *Macrophomina* spp. currently occurring in Brazil are scarce. Therefore, this work aims to investigate the characterization and pathogenicity of *M. phaseolina*, *M. pseudophaseolina* and *M. euphorbiicola* isolates obtained from *T. portulacastrum* and *B. diffusa* collected in northeast Brazil, regarding: i) the effect of temperature and salinity on mycelial growth, ii) their pathogenicity on melon and watermelon seedlings, and iii) sensitivity to the carbendazim fungicide.

MATERIAL AND METHODS

Fungal isolates

In this study, eight *Macrophomina* isolates were used. Six isolates of three species of *Macrophomina* (*M. phaseolina* – CMM4738 and CMM4763, *M. pseudophaseolina* – CMM4771 and CMM4801; and *M. euphorbiicola* – CMM4867 and CMM4868) from asymptomatic roots of the weed species *T. portulacastrum* and *B. diffusa*, collected in melon and watermelon fields located in the Rio Grande do Norte (RN) and Ceará (CE) states, northeastern Brazil; and two isolates of M. phaseolina, collected from a melon (CMM1531) and watermelon (MC01) roots, were used as positive controls in the experiments (Table 1).

The CMM4867 and CMM4868 isolates of *M. euphorbiicola* and CMM1531 and MC01 of *M. phaseolina* were identified through phylogenetic inference based on the partial sequence of the translation elongation factor-1*alpha* (*tef-1α*) using the primers EF728F and EF986R (CARBONE; KOHN, 1999). The others isolates of *Macrophomina* spp. were identified by Negreiros et al. (2019).

The isolates (CMM4738, CMM4763, CMM4771, CMM4801, CMM4867, CMM4868 and CMM1531) were deposited in the Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM) at the Universidade Federal Rural de Pernambuco (Recife, Pernambuco, Brazil). The isolate (MC01) was deposited in the culture collection of plant pathogenic fungi of Universidade Federal Rural do Semi-Árido (Mossoró, Rio Grande do Norte, Brazil). All isolates were hyphal□tipped and, then, they were stored on sandy□organic substrate and Castellani's method with distilled water (NEGREIROS et al., 2019).

Table 1. List of *Macrophomina* species used in this study.

Macrophomina species	Isolate number[a]	Host[c]	Location[b]	GenBank Accession Numbers
M. phaseolina	CMM4738	TP	Brazil, CE, Icapuí	MH373461
	CMM4763	BD	Brazil, RN, Mossoró	MH373451
M. pseudophaseolina	CMM4771	TP	Brazil, RN, Assú	MH373471
	CMM4801	BD	Brazil, RN, Assú	MH373517
M. euphorbiicola	CMM4867	TP	Brazil, RN, Assú	MH712509
	CMM4868	BD	Brazil, RN, Assú	MH712510
M. phaseolina (Positive Control - PC)	CMM1531	CM	Brazil, RN, Mossoró	MN136199
	MC01	CL	Brazil, CE, Icapuí	MN136200

[a]Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM) of the Universidade Federal Rural de Pernambuco (Recife, PE, Brazil). [b]Ceará state (CE) and Rio Grande do Norte state (RN). [c] TP - *Trianthema portulacastrum*. BD - *Boerhavia diffusa*. CM - *Cucumis melo*. CL - *Citrullus lanatus.*

Effects of temperature on mycelial growth of *Macrophomina*

The mycelial growth gate (MGR) was measured in colonies grown in Petri plates containing potato-dextrose-agar (PDA) (MAYEK-PÉREZ; LÓPEZ-CASTAÑEDA; ACOSTA-GALLEGOS, 1997). Mycelial plugs (8 mm in diameter) obtained from the growing edge of 7-day-old colonies of isolates were placed in the centre of each Petri plate (one plug per plate), which were then incubated at the temperatures of 25, 30, 35, 40 and 45 ± 1 ^{o}C, in the dark, for seven days. The colony diameter of each isolate for all temperatures was measured daily along two perpendicular axes until the colony reached the edge of the Petri plate and the data were used to calculate the MGR of the colony (cm day^{-1}).

Effect of salinity on mycelial growth of *Macrophomina* spp.

The effect of salinity on mycelial growth of all isolates *in vitro* was analysed using PDA adjusted to the following sodium chloride (NaCl) concentrations: 0, 250, 500, 750 and 1000 mM (corresponding to 0.82, 3.70, 5.57, 7.59 and 9.36 dS m^{-1}, respectively). All concentrations were sterilized prior to its use in autoclave for 15 min, at 121 ºC and 1 Bar. Mycelial discs (8 mm in diameter) taken from 7-day old fungal colonies of each isolate were transferred to Petri plates containing PDA adjusted to each NaCl concentration, and incubated at 30 ºC in the dark. The average diameter of the fungal colony was measured daily and data were used to calculate the MGR of the colony.

Pathogenicity of *Macrophomina* spp. on melon and watermelon seedlings

The pathogenicity of *Macrophomina* spp. was evaluated on melon "Gladial" and watermelon "Crimson sweet" seedlings. Melon and watermelon seeds were germinated in pots containing Tropstrato HT® commercial substrate previously autoclaved. The seedlings were daily irrigated to drainage with tap water. For inoculation the toothpick method was used, because of the easy multiplication of inoculum and fast inoculation (NEGREIROS et al., 2019). Melon and watermelon seedlings were inoculated 10 days after sowing (DAS) by inserting the toothpicks colonized with mycelia and microsclerotia of the corresponding isolate in each hypocotyl, 1 cm above the soil. Non-infested and autoclaved toothpicks were used as negative controls.

The inoculated seedlings were maintained in a greenhouse at an average temperature of 35 ± 2 °C, under natural daylight conditions using a completely randomized experimental design, with five replicates per treatment (isolate). Thirty days after inoculation, disease incidence was determined as the total number of infected plants from each *Macrophomina* species and expressed as a percentage. The aggressiveness of the isolates was assessed as disease severity using a modified version of the rating scale described by Ambrósio et al. (2015), where, 0 = symptomless, 1 = less than 3% of shoot tissues infected, 2 = 3–10% of shoot tissues infected, 3 = 11–25% of shoot tissues infected, 4 = 26–50% of shoot tissues infected and 5 = more than 50% of shoot tissues infected.

Sensitivity of *Macrophomina* spp. to carbendazim

The sensitivity of the three *Macrophomina* species to the fungicide carbendazim (methyl-2-benzimidazole carbamate, benzimidazoles chemical group) was determined in PDA supplemented with carbendazim, and the MGR was evaluated. The treatments included five levels of carbendazim concentration: 0.01, 0.10, 1, 10 and 100 mg L^{-1} active ingredient (a.i.) (TONIN et al., 2013). Petri

plates containing PDA without fungicide were used as the control (0 mg L^{-1}). A 7-day old mycelial plug (8 mm in diameter) from each isolate of *Macrophomina* was placed in the centre of the Petri plates containing PDA supplemented with the concentrations of the fungicide, and incubated in the dark at 30 °C. The radial growth (diameter) of each colony was measured daily in two perpendicular directions, until the colony reached the edge of the Petri plate and the mean diameter of the colony was obtained.

Relationships between adaptability components and pathogenicity of *Macrophomina* spp.

To establish the relationship between adaptability components and pathogenicity of *Macrophomina* spp., a heatmap and principal component analysis (PCA) were performed. The standardized Euclidean distance between species pairs was used to construct a heatmap (dendrogram) by unweighted paired group method with arithmetic averages (UPGMA) (KOLDE, 2022) and the PCA (LE; JOSSE; HUSSON, 2008) was performed by correlation matrix, both using the software "R" (R CORE TEAM, 2022).

Statistical analysis

All experiments were conducted in a completely randomized design, with five repetitions of each isolate. All experiments were repeated. Preliminary ANOVAs were performed to determine if there were significant differences between the repetitions of the experiments and whether the data could be combined. The MGR means of temperature and NaCl concentrations of all isolates were subjected to a regression analysis using TableCurve 2D v.5.01 (SYSTAT Software Inc.). For each temperature and NaCl concentration, the means of the isolate were compared by Tukey test at the 5% significance level using the software "R" (R CORE TEAM, 2022). Differences in incidence and severity caused by *Macrophomina* species for melon and watermelon were analysed with the non-parametric Kruskal-Wallis test at the probability level of 5% ($p < 0.05$) using the software "R" (R CORE TEAM, 2022). The MGR of the fungicide was used to determine the effective concentration for 50% reduction in growth – EC_{50} (mg L^{-1} of a.i. inhibiting MGR by 50%) for each isolate of *Macrophomina* and the method was based on linear regression by plotting values of Log-Probit.

For all experiments, no significant effect of the experiment repetitions (ANOVA, $p > 0.05$) was found, thus the data were combined.

RESULTS AND DISCUSSION

Effects of temperature on mycelial growth of *Macrophomina* spp.

In the temperature study, *Macrophomina* spp. showed statistically significant effects ($p \leq 0.05$) for fungal MGR. The optimum temperature for the three *Macrophomina* spp. ranged from 27.18 ºC (CMM4771 – *M. pseudophaseolina*) to 31.80 ºC (CMM4763 – *M. phaseolina*), being the mean MGR of the isolates 29.57 ºC (Table 2). The *M. phaseolina* isolates (CMM4738, CMM4763, CMM1531 and MC01) showed the optimum temperature of 28.51, 31.80, 29.10 and 28.09 ºC, respectively. However, the optimum temperature for *M. pseudophaseolina* isolates (CMM4771 and CMM4801) was 27.18 and 31.32 ºC, respectively, and for *M. euphorbiicola* isolates (CMM4867 and CMM4868) was 28.89 and 31.65 ºC, respectively. The MGR of the *M. euphorbiicola* isolates (CMM4867 and CMM4868) and *M. phaseolina* (CMM1531) at 25 ºC and 30 ºC was significantly higher than those of other isolates. At 35 ºC and 40 ºC, the MGR of the isolate CMM4868 (*M. euphorbiicola*) was significantly higher than other isolates. No growth was observed at 45 °C for any of the three *Macrophomina* species isolates evaluated in this study after seven days of incubation.

In this study, optimal growth temperatures for the isolates of *Macrophomina* spp. ranged between 27.18 and 31.80 °C, which differs from previously published results by Sarr et al. (2014). These authors studying the genetic diversity of *M. phaseolina* in Senegal, reported for *M. phaseolina* and *M. pseudophaseolina* optimal growth temperature in the range of 30 to 36 ºC and there was still growth at 40 ºC. In a previous study, Cardona (2006) reported the ideal temperature for *M. phaseolina* in Venezuela as in the range of 28 to 32 ºC, our results are within this range. Previously, Csöndes (2012) reported that the most favourable temperature interval for the development of *M. phaseolina* isolates in Hungary was from 25 to 35°C. The number of studies concerning optimal temperatures for maximal growth of *M. pseudophaseolina* and *M. euphorbiicola* is even more limited in relation to the importance that it can have for effective management of the disease. The occurrence of *Macrophomina* spp. in growing areas of melon and watermelon in the northeastern semi-arid region might be related to the hot and dry climate of the region (INMET, 2022), with average annual temperatures ranging from 23.2–33.8 ºC, consequently, *Macrophomina* is one of the most frequent root pathogens isolated from symptomatic melon and watermelon plants.

Table 2. Optimum temperature and mycelial growth at 25, 30, 35 and 40 ºC of *Macrophomina* spp. from northeastern Brazil.

Macrophomina species	Isolates	Temperature				
		Optimum temperature (°C)	Mycelial Growth Rate (cm d^{-1})[b]			
			25ºC	30ºC	35ºC	40ºC
M. phaseolina	CMM4738	28.51	1.07 bc	1.39 b	0.70 d	0.35 cde
	CMM4763	31.80	0.63 e	1.34 b	1.59 b	0.67 b
M. pseudophaseolina	CMM4771	27.18	0.83 cde	0.88 d	0.44 e	0.22 e
	CMM4801	31.32	0.74 de	0.86 d	0.97 c	0.57 b
M. euphorbiicola	CMM4867	28.89	1.37 a	1.93 a	0.97 c	0.48 bcd
	CMM4868	31.65	1.20 ab	2.05 a	1.94 a	1.34 a
M. phaseolina (PC[a])	CMM1531	29.10	1.37 a	2.05 a	1.03 c	0.51 bc
M. phaseolina (PC[a])	MC01	28.09	0.91 cd	1.09 c	0.54 de	0.27 de
Mean		29.57	1.01	1.45	1.02	0.55
CV (%)		-	11.71	5.79	9.28	16.41

[a] PC (Positive control). [b] Values with the same letter within a column are not significantly different according to the Tukey test at 5% probability.

Effect of salinity on mycelial growth of *Macrophomina* spp.

The mean NaCl concentrations of the *Macrophomina* spp. isolates were subjected to a regression analysis with a correlation coefficient $R^2 > 0.99$, and showed statistically significant effects ($p < 0.05$). The EC_{50} for all *Macrophomina* isolates ranged from 103.76 (CMM4868 – *M. euphorbiicola*) to 315.25 mM (CMM4801 – *M. pseudophaseolina*), with a mean EC_{50} of the isolates of 192.97 mM (Table 3). The *M. phaseolina* isolates showed EC_{50} of 175.12 (CMM4738), 132.09 (CMM4763), 219.81 (CMM1531) and 193.06 mM (MC01). However, the EC_{50} for *M. pseudophaseolina* isolates (CMM4771 and CMM4801) was 194.08 and 315.25 mM, respectively, and for *M. euphorbiicola* isolates (CMM4867 and CMM4868) was 210.56 and 103.76 mM, respectively. Statistically significant effects of the isolates of *Macrophomina* spp. for each NaCl concentration on MGR were observed ($p < 0.05$). At 0 mM (control), the mean MGR of the isolates was 1.22 cm d^{-1} and the values of this variable ranged from 0.83 (CMM4771) to 2.05 cm d^{-1} (CMM4868). At 250 mM, the mean MGR was 0.35 cm d^{-1} and the values ranged between 0.21 (MC01) and 0.64 cm d^{-1} (CMM4867). This concentration showed a 71.31% reduction in the mean MGR in relation to the concentration of 0 mM (control). At 500 mM, the mean MGR was 0.15 cm d^{-1}, the values ranged between 0.08 (CMM4771) and 0.24 cm d^{-1} (CMM1531), and 87.70% of reduction of the mean MGR in relation to the concentration of 0 mM. At 750 mM, the mean MGR was 0.09 cm d^{-1}, the values ranged between 0.02 (CMM4868) and 0.21 cm d^{-1} (CMM1531), and this concentration showed 92.62% of reduction in the MGR in relation to the concentration of 0 mM. At 1000 mM, the mean MGR was 0.05 cm d^{-1}, the values ranged between 0.01 (CMM4867) and 0.09 cm d^{-1} (CMM1531), and showed 95.90% of reduction of the MGR in relation to the concentration of 0 mM.

Sodium chloride reduced *in vitro* growth of *Macrophomina* species, particularly in isolate CMM4868 (*M. euphorbiicola*); while the isolate CMM4801 (*M. pseudophaseolina*) was the most tolerant to sodium chloride. Sodium chloride caused the greatest negative effects on the development of *Macrophomina* spp. Variations in response to salinity in the mycelial growth of fungi are not unusual, but for the species *M. pseudophaseolina* and *M. euphorbiicola* these data are scarce, therefore, the data from this study show this variation between isolates and within the *Macrophomina* genus. Similar results have been reported in *M. phaseolina* by Cervantes-García et al. (2003) and *Fusarium solani* (Mart.) Sacc. (PALACIOS et al., 2014). Negative effect by NaCl on *Macrophomina* spp. growth may be related to the modification of water availability in the PDA medium; therefore, the osmotic potential is lower in each fungal cell compared with the conditions of the PDA. The NaCl present in the PDA medium traps water molecules, therefore water will not be available to the isolates of *Macrophomina*. The energy spent by the fungus in order to obtain water molecules from the medium is increased as the NaCl concentrations in the PDA increase. Thus, the fungus is obliged to reduce its growth rate under *in vitro* conditions (CERVANTES-GARCÍA et al., 2003).

Table 3. Effective concentration for 50% reduction in growth (EC_{50}) and mean mycelial growth at 0, 250, 500, 750, and 1000 mM of sodium chloride of *Macrophomina* spp. from northeastern Brazil.

Macrophomina species	Isolates	Salinity						
		Regression equation[b]	EC_{50} (mM)	Mycelial Growth Rate (cm d^{-1}) [c]				
				0	250	500	750	1000
M. phaseolina	CMM4738	y = 0.0668-0.0039x	175.12	1.07 c	0.35 bcd	0.16 b	0.08 bcd	0.05 b
	CMM4763	y = 0.2849-0.0052x	132.09	1.34 b	0.23 cd	0.11 cd	0.07 bcde	0.03 bcd
M. pseudophaseolina	CMM4771	y=-0.2118-0.0034x	194.08	0.83 d	0.27 cd	0.08 d	0.11 b	0.05 b
	CMM4801	y=-0.0151-0.0034x	315.25	0.86 d	0.38 bc	0.15 bc	0.05 cde	0.04 bc
M. euphorbiicola	CMM4867	y=0.3214-0.0034x	210.56	1.37 b	0.64 a	0.18 b	0.05 de	0.01 d
	CMM4868	y=0.7127-0.0066x	103.76	2.05 a	0.26 cd	0.14 bc	0.02 e	0.02cd
M. phaseolina (PC[a])	CMM1531	y=0.2928-0.0031x	219.81	1.37 b	0.50 ab	0.24 a	0.21 a	0.09 a
M. phaseolina (PC[a])	MC01	y=-0.1164-0.0034x	193.06	0.91 cd	0.21 d	0.14 bc	0.10 bc	0.08 a
Mean			192.97	1.22	0.35	0.15	0.09	0.05
CV (%)		-	-	6.64	19.49	12.16	23.84	21.92

[a] PC (Positive control).
[b] y = mycelial growth rate; x = salinity concentration.
[c] Values with the same letter within a column are not significantly different according to the Tukey test at 5% probability.

Pathogenicity of *Macrophomina* spp. on melon and watermelon

All isolates of *Macrophomina* were pathogenic to melon seedlings, but for the watermelon seedlings only the isolates CMM4801 (*M. pseudophaseolina*), CMM4763 and MC01 (*M. phaseolina*) were pathogenic (Table 4). The results showed that the incidence and severity of disease presented significant differences ($p \leq 0.05$) for the isolates of *M. phaseolina*, *M. pseudophaseolina* and *M. euphorbiicola*, in each culture. In melon seedlings, the isolates CMM4738, CMM4763 and CMM1531 (*M. phaseolina*) were statistically different to the isolate CMM4868 (*M. euphorbiicola*) for disease incidence, presenting the highest averages 100%, 100% and 100%, respectively. However, for disease severity, only isolates CMM4771 (*M. pseudophaseolina*) and CMM4868 (*M. euphorbiicola*) differed from CMM1531 (*M. phaseolina*), the other isolates did not differ from each other. The isolates of *M. pseudophaseolina* and *M. euphorbiicola* showed intermediate values ranging from 0.8 (CMM4771 and CMM4868) to 3.8 (CMM4867) for severity, and from 20% (CMM4868) to 80% (CMM4801 and CMM4867) for disease incidence to melon. In watermelon seedlings, the isolates CMM4763 (*M. phaseolina*) and CMM4801 (*M. pseudophaseolina*) were not statistically different from the MC01 (*M. phaseolina* – PC) for disease incidence and severity, the isolates showed the same incidence and severity values of 40% and 0.4, respectively, to watermelon.

Table 4. Disease severity and incidence induced to *Cucumis melo* and *Citrullus lanatus* seedlings by *Macrophomina* spp. from northeastern Brazil.

Macrophomina species	Isolates	*Cucumis melo*				*Citrullus lanatus*			
		Disease incidence (%)		Disease severity		Disease incidence (%)		Disease severity	
		Rank[b]	Mean	Rank[b]	Mean	Rank[b]	Mean	Rank[b]	Mean
M. phaseolina	CMM4738	22.5 b	100	25.6 ab	4.8	13.5 a	0	13.5 a	0.0
	CMM4763	22.5 b	100	25.1 ab	4.6	20.5 ab	40	19.9 ab	0.4
M. pseudophaseolina	CMM4771	12.0 ab	40	8.1 a	0.8	13.5 a	0	13.5 a	0.0
	CMM4801	19.0 ab	80	10.8 ab	1.4	20.5 ab	40	19.9 ab	0.4
M. euphorbiicola	CMM4867	19.0 ab	80	21.0 ab	3.8	13.5 a	0	13.5 a	0.0
	CMM4868	8.5 a	20	7.9 a	0.8	13.5 a	0	13.5 a	0.0
M. phaseolina (PC[a])	CMM1531	22.5 b	100	27.5 b	5.0	-	-	-	-
M. phaseolina (PC[a])	MC01	-	-	-	-	31.0 b	100	32.2 b	3.4
	Mean	-	74.3	-	3.0	-	26.0	-	0.6
	χ^{2} [d]	15.7	-	24.5	-	21.8	-	23.6	-

[a] Positive control (PC).
[b] χ^2 = chi-square value significant at 5% by Kruskal-Wallis test.

The pathogenicity test demonstrated that *M. phaseolina*, *M. pseudophaseolina* and *M. euphorbiicola* are pathogenic to melon with *M. phaseolina* exhibiting a higher level of aggressiveness. However, for watermelon, only CMM4801 (*M. pseudophaseolina*), CMM4763 and MC01 (*M. phaseolina*) isolates were able to cause disease with MC01 isolate exhibiting a higher level of virulence in the experiment. In this study, *M. euphorbiicola* isolates did not cause disease in watermelon. In a previous study, Ndiaye et al. (2015) investigating the pathogenicity of *M. phaseolina* and *M. pseudophaseolina* on three varieties of cowpea, observed that both species of *Macrophomina* induced disease. Negreiros et al. (2019) studying the pathogenicity of *M. phaseolina* and *M. pseudophaseolina* from weed species on melon seedlings revealed that all *M. phaseolina* isolates inoculated were able to cause disease to melon seedlings, but only some *M. pseudophaseolina* isolates were able to infect them.

Sensitivity of *Macrophomina* spp. to carbendazim

The effects of different concentrations of carbendazim on MGR of *Macrophomina* are shown in Table 5. Regression equations for log-Probit were adjusted and the EC_{50} values were calculated. The mean EC_{50} was 0.034 mg L^{-1} a.i. and the values of this variable ranged from 0.003 (*M. euphorbiicola*) to 0.089 (*M. pseudophaseolina*) mg L^{-1} a.i. of carbendazim (Table 5). The most sensitive isolates to the fungicide carbendazim were CMM4868, CMM4867 (*M. euphorbiicola*) and CMM1531 (*M. phaseolina*) with EC_{50} of 0.003, 0.012 and 0.012 mg L^{-1} a.i., respectively. In contrast, the most tolerant species was *M. pseudophaseolina* (CMM4771 and CMM4801) with EC_{50} of 0.060 and 0.089 mg L^{-1} a.i., respectively.

Table 5. Regression equation and effective concentration for 50% reduction in growth (EC_{50}) for log-Probit analysis by fungicide carbendazim by *Macrophomina* spp. from northeastern Brazil.

Macrophomina species	Isolates	Fungicide Carbendazim	
		Regression equation[b]	EC_{50}[c] (mg L^{-1} a.i.)
M. phaseolina	CMM4738	y=18.2693252040x+75.2023275931	0.042
	CMM4763	y=15.4255583900x+77.5926546017	0.016
M. pseudophaseolina	CMM4771	y=18.7431597070x+72.8887478025	0.060
	CMM4801	y=19.6776603899x+70.6840985836	0.089
M. euphorbiicola	CMM4867	y=15.3423755467x+79.6945427294	0.012
	CMM4868	y=13.2169312169x+82.5211640212	0.003
M. phaseolina (PC[a])	CMM1531	y=14.5357142857x+77.8888888889	0.012
M. phaseolina (PC[a])	MC01	y=16.6386225656x+76.0844032340	0.027
	Mean	-	0.034
	χ^{2} [d]	-	-

[a] Positive control (PC).
[b] y = percentage of mycelial growth inhibition for log-Probit analysis; and x = fungicide concentration.
[c] Calculated by the concentration equation (mg L^{-1}) for logProbit analysis.

The carbendazim fungicide test of the three *Macrophomina* species showed that the EC_{50} for all the isolates ranged from 0.003 to 0.089 mg L^{-1}. These isolates were considered sensitive to carbendazim (EC_{50} < 0.1 mg L^{-1}). Edgington, Khew and Barrow (1971) proposed the following criteria to rank the fungitoxicity of a fungicidal substance: EC_{50} < 1 mg L^{-1} = highly fungitoxic, EC_{50} of 1–50 mg L^{-1} = moderately fungitoxic and EC_{50} > 50 mg L^{-1} = non-toxic. Thus, in this study, carbendazim was considered a highly fungitoxic chemical for all isolates of the studied species. Only a few studies have reported the sensitivity of *M. phaseolina* to carbendazim, but there are no studies in the literature that show the relationship of *M. pseudophaseolina* and *M. euphorbiicola* with carbendazim. This agrees with other studies that reported some degree of sensibility among *M. phaseolina* isolates to carbendazim (CHAUHAN, 1988). The carbendazim EC_{50} values obtained in the present study were consistent with that of a previous study involving a Brazilian isolate of *M. phaseolina* from *G. max* (TONIN et al., 2013). Carbendazim belongs to the benzimidazole systemic fungicides group and is a

potent inhibitor of tubulin polymerization and exerts its antifungal activity by targeting the β-tubulin subunit of the microtubules, which results in the arrest of microtubule formation and a failure in cell division, subsequently leading to cell death (FRAC, 2021). In relation to its chemical control, in Brazil, there are no fungicides registered for this pathogen in melon and watermelon crops (AGROFIT, 2022). In the present study, the EC_{50} of the active ingredient carbendazim showed it to be a viable alternative for controlling *Macrophomina* spp. However, field studies will be necessary to evaluate the efficiency of this fungicide.

Relationships between adaptability components and pathogenicity of *Macrophomina* species

Cluster analysis using the UPGMA method was used to group *Macrophomina* species as a function of the Euclidean distance obtained from their adaptability components and pathogenicity (Figure 1). Three groups of *Macrophomina* species were formed. The first two groups were unitary, the first consisting of CMM4868 (*M. euphorbiicola*) and the second CMM1531 (*M. phaseolina*). The isolate CMM4868 was less sensitive to the fungicide since it had greater growth in almost all concentrations applied. However, it was more sensitive to salinity and less pathogenic to melon and watermelon. The CMM1531 isolate was more sensitive to the fungicide but its growth was little affected by salinity and temperature, mainly up to 30 ºC. It was pathogenic to melon.

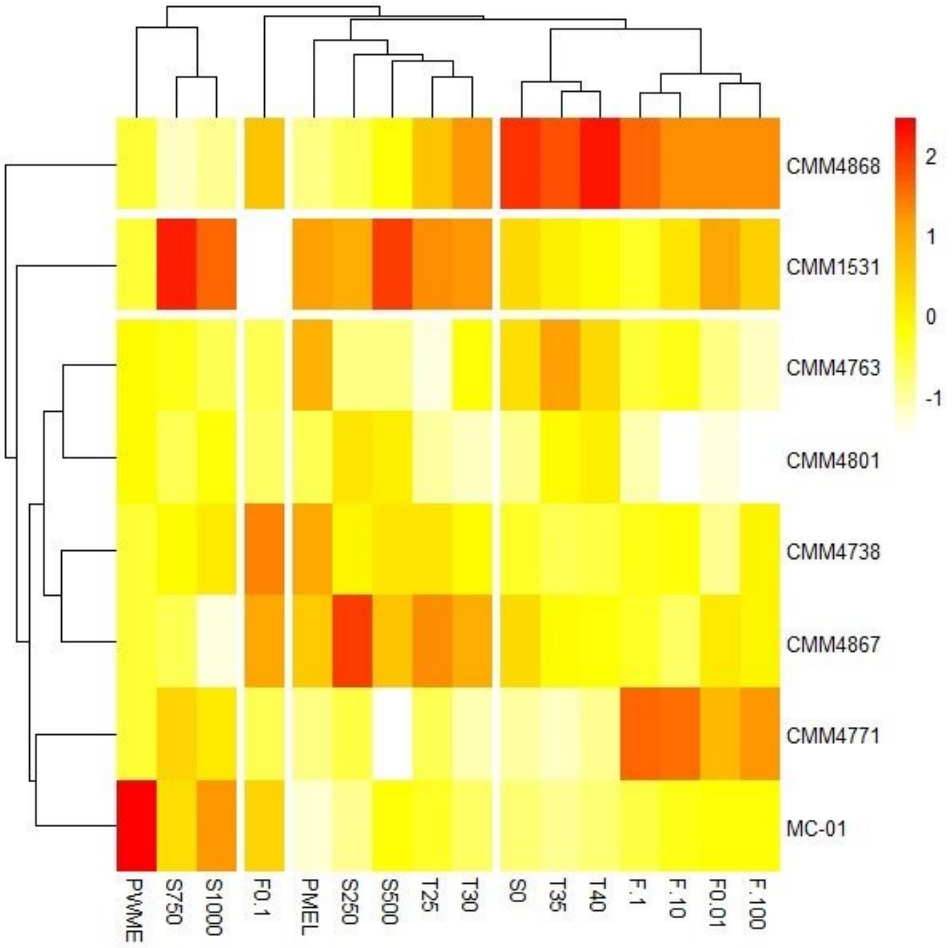

Figure 1. Heatmap showing the grouping of *Macrophomina* isolates when analyzing the temperature, salinity, pathogenicity and fungicide (PWME, watermelon pathogenicity; S750, 750 mM salinity; S1000, 1000 mM salinity; F0.1, fungicide at the concentration 0.1 mg L^{-1} a.i.; PMEL, melon pathogenicity; S250, 250 mM salinity; S500, 500 mM salinity; T25, the temperature at 25 ºC; T30, the temperature at 30 ºC; S0, salinity at 0 mM; T35, the temperature at 35 ºC; T40, the temperature at 40 ºC; F.1, fungicide at a concentration of 1 mg L^{-1} a.i.; F.10, fungicide at a concentration of 10 mg L^{-1} a.i.; F0.01, fungicide at a concentration of 0.01 mg L^{-1} a.i.; F.100, fungicide at concentration 100 mg L^{-1} a.i.). The dendrogram and order were determined using the functions of calculating the distance matrix (dist) and hierarchical cluster (hclust) in R. The red colours indicate higher rates of mycelial growth (temperature and salinity), a higher percentage of growth inhibition (fungicide) and higher disease severity (pathogenicity), while the white colours indicate lower growth rates of mycelial growth (temperature and salinity), a lower percentage of growth inhibition (fungicide) and lower disease severity (pathogenicity).

The third group brought together the other isolates that, in general, have their growth less influenced by the adaptability components (Figure 1). However, there was variation within the group. The isolate CMM4867 (*M. euphorbiicola*) showed similar behavior to the isolate CMM1531, that is, greater sensitivity to the fungicide and with greater growth in salinity up to 500 mg L^{-1} (S500) and temperatures up to 30 ºC (T30). The isolate CMM4771 (*M. pseuphaseolina*) was less sensitive to the fungicide but affected by temperature and salinity. The isolate MC01 (*M. phaseolina*), as expected, was the most pathogenic to watermelon.

From the principal component analysis (PCA), it can be noted that the first component explains 33.40% of the data variability, while the second explains 24.06 %, totalling 57.46 % (Figure 2). The effects of the fungicide, especially the concentrations 1 mg L^{-1} and 10 mg L^{-1} , plus the higher temperatures, are the variables most associated with the first major component (CP1). On the other hand, salinity, especially concentrations 250 (S250) and 500 (S500), plus lower temperatures (25 and 30 ºC), are the variables most associated with the second main component (Figure 2).

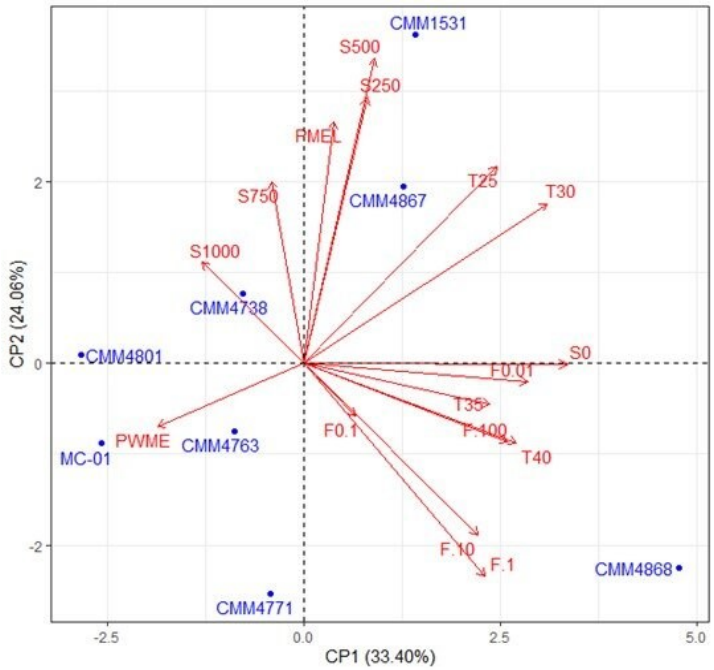

Figure 2. Principal component analysis (PCA) of *Macrophomina* isolates with their adaptability components and pathogenicity (PWME, watermelon pathogenicity; S750, 750 mM salinity; S1000, 1000 mM salinity; F0.1, fungicide at the concentration 0.1 mg L^{-1} a.i.; PMEL, melon pathogenicity; S250, salinity at 250 mM; S500, salinity at 500 mM; T25, temperature at 25 ºC; T30, temperature at 30 ºC; S0, salinity at 0 mM; T35, temperature at 35 ºC; T40, temperature at 40 ºC; F.1, fungicide at the concentration 1 mg L^{-1} a.i.; F.10, fungicide at the concentration 10 mg L^{-1} a.i.; F0.01, fungicide at the concentration 0.01 mg L^{-1} a.i.; F.100, fungicide at a concentration of 100 mg L^{-1} a.i.).

Regarding the distribution of species in relation to the adaptability components and pathogenicity, a result similar to that observed for the cluster analysis was observed. The CMM4868 isolate had greater growth when associated with different concentration of fungicide and higher temperatures but growth was reduced by lower salinity and temperatures. The CMM1531 isolate had greater growth in salinity concentration and was more affected by the fungicide. The isolate CMM4867 showed greater sensitivity to the fungicide but with greater growth in salinity and temperatures up to 30 ºC (T30). The isolate CMM4741 (*M. pseudophaseolina*) was less sensitive to the fungicide, but affected by temperature and salinity. The isolate MC01 stood out due to its greater pathogenicity to watermelon.

This work reports for the first time the association of *M. euphorbiicola* with asymptomatic roots of *T. portulacastrum* and *B. diffusa* weeds, which are common in the main Brazilian producing and exporting regions of melon and watermelon. The information generated in this research will increase knowledge about the epidemiology of disease and may help to predict the risk of *Macrophomina* root rot.

CONCLUSION

Macrophomina spp. used in these experiments showed growth variations “in vitro” for

different temperatures, salinity and fungicide concentrations. All *Macrophomina* spp. used in these experiments were pathogenic to the tested melon and watermelon cultivars with the exception of the *M. euphorbiicola* isolates that did not cause damage to watermelon. Additional studies with new hybrids of these cultures should be carried out as a way to genetically manage these pathosystems in the studied region.

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