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1	Characterization of adaptability components of Brazilian isolates of Macrophomina
2	pseudophaseolina
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13	
14	Abstract
15	Macrophomina pseudophaseolina is a new Macrophomina species reported on
16	different crop and weed species in Brazil, India and Senegal, but to date there are no
17	studies about its adaptability components. In this work a collection of $62 M$ .
18	pseudophaseolina isolates obtained from roots of the weed species Trianthema

19 *portulacastrum* and *Boerhavia diffusa* collected in Northeastern Brazil, was used to: i) 20 study the effect of temperature and salinity on mycelial growth, ii) to determine their 21 sensitivity to the fungicide carbendazim, and iii) to assess their aggressiveness on melon 22 and watermelon seedlings. Results showed variability among *M. pseudophaseolina* 23 isolates. The optimum temperature for mycelial growth ranged between 26.4 and 38.1°C. 24 NaCl reduced the *in vitro* growth of all isolates, which were also highly sensitive to the 25 fungicide carbendazim, exhibiting EC<sub>50</sub> values ranging from 0.013 to 0.089 mg L<sup>-1</sup> a.i.

26	Disease severity values on melon and watermelon seedlings showed that $M$ .										
27	pseudophaseolina isolates were more aggressive in melon than in watermelon.										
28	Information about adaptability components of M. pseudophaseolina obtained in this study										
29	could be incorporated on breeding programs for melon and watermelon crops.										
30											
31	KEYWORDS: Boerhavia diffusa, fungicide sensitivity, mycelial growth, pathogenicity										
32	test, Trianthema portulacastrum										
33											
34	1 INTRODUCTION										
35											
36	The genus Macrophomina (Botryosphaeriaceae, Ascomycota) has a worldwide										
37	distribution and it has been described in more than 750 host plant species, many of which										
38	are economically important crops (Farr and Rossman 2019).										
39	For long time M. phaseolina was the only species included in this genus (Farr and										
40	Rossman 2019). However, recent studies have revealed the existence of three new										
41	Macrophomina species: M. pseudophaseolina (Sarr et al. 2014), M. euphorbiicola										
42	(Machado et al. 2019), and M. vaccinni (Zhao et al. 2019).										
43	Macrophomina phaseolina is a well-known plant pathogen, causing several types										
44	of diseases, such as <i>damping-off</i> , seed rot, root rot, charcoal rot and gray stem rot, being										
45	more aggressive in subtropical and tropical countries with semi-arid climate (Dhingra and										
46	Sinclair 1978; Wrather et al. 2001; Gupta et al. 2012). Moreover, M. phaseolina can										
47	survive in soil for many years, in seeds and/or crop residues, by forming microsclerotia										
48	as resistance structures (Dhingra and Sinclair 1978; Gupta et al. 2012).										
49	The three recently described Macrophomina species, M. pseudophaseolina, M.										
50	euphorbiicola, and M. vaccinni, are examples about how advances in fungal phylogenetic										

51 studies are allowing the identification of new species in fungal populations worldwide 52 (Sarr et al. 2014; Machado et al. 2019; Zhao et al. 2019). The species M. 53 pseudophaseolina was described in Senegal, in 2014, associated with Vigna unguiculata, 54 Arachis hypogaea, Hibiscus sabdarifa and Abelmoschus esculentus (Sarr et al., 2014). In 55 Brazil, this new Macrophomina species was first reported on A. hypogaea, Gossypium 56 hirsutum, Ricinus communis, Manihot esculenta, and also associated with Jatropha 57 curcas seeds (Brito et al. 2019; Machado et al. 2019). Later M. pseudophaseolina was 58 also found on roots of two weed species, Trianthema portulacastrum and Boerhavia 59 diffusa, which are prevalent in melon fields located at the main cucurbit producing and 60 exporting regions of northeastern Brazil (Negreiros et al. 2019). Recently, M. 61 pseudophaseolina has been reported on Coleus forskohlii in India (Mastan et al. 2019).

62 For any new fungal species, studies about its adaptability components have 63 become important, as they allow the characterization of isolates collections (Lannou 64 2012). Moreover, competitive capacity between fungal populations can be indirectly 65 inferred through components of adaptability (Zhan and McDonald 2013). Since 66 adaptability is relative, it should be estimated by measuring characters that restrict some 67 adaptative advantage among individuals. Phenotypic markers such as mycelial growth at 68 different temperatures, reproductive potential, salinity and fungicide sensitivity and 69 aggressiveness are useful to evaluate the variability of adaptability in fungal plant 70 pathogen populations (Antonovics and Alexander 1989; Lannou 2012).

To date, there are no studies about adaptability components of *M. pseudophaseolina*. Therefore, this work aims to investigate the adaptability components of a collection of 62 *M. pseudophaseolina* isolates obtained from roots of the weed species *T. portulacastrum* and *B. diffusa* collected in Northeastern Brazil, with the following objectives: i) to study the effect of temperature and salinity on mycelial growth, ii) to determine their sensitivity to the fungicide carbendazim, and iii) to assess theiraggressiveness on melon and watermelon seedlings.

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## 79 2 MATERIALS AND METHODS

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## 81 **2.1 Fungal isolates**

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In this study, 62 M. pseudophaseolina isolates obtained in 2015 from 83 asymptomatic roots of the weed species T. portulacastrum and B. diffusa, collected in 84 melon and watermelon fields located at, Mossoró and Assú (Rio Grande do Norte-RN 85 State), and Icapuí (Ceará-CE State), were used (Table 1). These weed species were 86 selected based on their prevalence in melon and watermelon fields in RN and CE, Brazil. 87 88 All isolates were hyphal-tipped and preserved using two different methods: on sandyorganic substrate and by Castellani's method with distilled water (Medeiros et al. 2015; 89 90 Gonçalves et al. 2016). These isolates were identified as belonging to the species M. 91 pseudophaseolina by Negreiros et al. (2019) and are deposited at the culture collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM) at the Universidade Federal Rural 92 de Pernambuco (Recife, Pernambuco, Brazil). Prior to use, all isolates were maintained 93 and grown in Petri plates with Potato-Dextrose-Agar (PDA; Merck KGaA, Darmstadt, 94 Germany) plates at 30°C in darkness. 95

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## 97 2.2 Effect of temperature on mycelial growth of *M. pseudophaseolina*

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99 The mycelial growth of all isolates was determined using cultures grown on PDA.100 Mycelial plugs (8 mm in diameter) obtained from the growing edge of 7-day-old colonies

were transferred to the center of PDA plates (one plug per plate), which were then 101 incubated in the dark at the temperatures of 25, 30, 35, 40, and  $45 \pm 1^{\circ}$ C for seven days, 102 103 with four replicates of each isolate and temperature combination. The colony diameter of 104 each isolate for all temperatures was daily measured along two perpendicular axes and the data were used to calculate the Mycelial Growth Rate (MGR) as mm per day (mm d<sup>-</sup> 105 <sup>1</sup>). The experiment was set up as a completely randomized design. The experiment was 106 conducted twice. A preliminary ANOVA analysis was performed to determine whether 107 108 there were significant differences between the two repetitions of the experiment and whether the data could be combined. Then, one-way analysis of variance (ANOVA) was 109 110 conducted with the data obtained from MGR. The optimum temperature for MGR of each isolate was plotted against temperature and a curve was fitted by a cubic polynomial 111 regression (y=a+bx+cx<sup>2</sup>+dx<sup>3</sup>) using TABLECURVE 2D v. 5.01 (SYSTAT Software, 112 113 Inc., 2002). The mean MGR of all isolates at each temperature were compared by Scott-114 Knott at the 5% significance level using SISVAR v. 5.6 (Ferreira 2011).

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# 116 **2.3 Effect of salinity on mycelial growth of** *M. pseudophaseolina*

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The effect of salinity on mycelial growth of all isolates in vitro was determined 118 on PDA adjusted to the following sodium chloride (NaCl) concentrations: 0, 250, 500, 119 120 750, and 1000 mM (Cervantes-García et al. 2003). Mycelial plugs (8 mm in diameter) obtained from the growing edges of colonies were transferred to the center of PDA plates 121 122 (one plug per plate) amended with the NaCl concentrations. The plates were sealed and incubated in the dark at 30°C for seven days. The experiment was set up as a completely 123 124 randomized design, with five replicates per each treatment. The average diameter of the fungal colony was daily measured and data were used to calculate the Mycelial Growth 125

Rate (MGR) of the colony (Mayek-Pérez et al. 1997). The experiment was conducted
twice. A preliminary ANOVA was performed to determine whether there were significant
differences between the two repetitions of the experiment and whether the data could be
combined. Then, one-way analysis of variance (ANOVA) was performed with MGR data,
and means were compared by Scott-Knott at the 5% significance level using SISVAR v.
5.6 (Ferreira 2011). The means of NaCl concentrations of all isolates were subjected to a
regression analysis using TABLECURVE 2D v. 5.01 (SYSTAT Software, Inc., 2002).

## 134 **2.4** Sensitivity of *M. pseudophaseolina* to the fungicide carbendazim

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The *M. pseudophaseolina* mycelial sensitivity to the fungicide carbendazim 136 (methyl-2-benzimidazole carbamate) was determined in vitro (Tonin et al. 2013). The 137 138 treatments included five levels of carbendazim concentration: 0.01, 0.10, 1, 10 and 100 mg L<sup>-1</sup> a.i. PDA plates without fungicide addition, were used as controls. A 7-day old 139 140 mycelial plug (8 mm diameter) from each M. pseudophaseolina isolate was placed in the 141 center of the Petri dishes containing each concentration of the fungicide and were incubated at  $30 \pm 1^{\circ}$ C in darkness for seven days. A complete randomized experimental 142 design was used, each treatment with five replicates per fungicide concentration and 143 144 isolate. The colony diameters (mm) were measured in two perpendicular directions, when 145 the fungal growth in the control treatment reached the edge of the plate. The experiment was conducted twice. A preliminary ANOVA was performed to determine whether there 146 147 were significant differences between the two repetitions of the experiment and whether the data could be combined. The SPSS version 22.0 was used to determine the half 148 maximal effective concentration (EC<sub>50</sub>) for the mycelial growth for each isolate of M. 149

- *pseudophaseolina*. The method was based on linear regression by plotting values of Log-Probit.
- 152

## 153 2.5 Pathogenicity and aggressiveness on melon and watermelon seedlings

154

The aggressiveness of the *M. pseudophaseolina* isolates was determined using a 155 toothpick method (Ambrósio et al. 2015) on melon and watermelon seedlings. Twelve 156 mm long toothpicks were placed, with the sharpened end up, in holes made in a 90 mm 157 diameter filter paper. The toothpicks were then placed in a Petri plates and autoclaved at 158 121°C for 30 min, for 2 days with an interval of 24 h. Then, 20 mL of potato dextrose 159 agar streptomycin (PDAS) was added to each toothpick-containing Petri plate. Once 160 solidified, each PDAS plate was inoculated with four mycelial plugs (8 mm in diameter) 161 of each isolate of *M. pseudophaseolina* and then were incubated at  $30 \pm 2^{\circ}$ C in the dark 162 163 for 7 days. Seeds of melon (cv. 'Gladial') and watermelon (cv. 'Crimson Sweet') were germinated in a 'Tropstrato HT<sup>®</sup>' commercial substrate previously autoclaved. The plants 164 were daily irrigated to drainage with tap water and were not fertilized during the 165 166 experiment. The seedlings of melon and watermelon were inoculated 10 days after sowing (DAS) by inserting the toothpicks colonized with mycelia and microsclerotia of the 167 168 corresponding isolate in each hypocotyl, one cm above the soil. Non infested and autoclaved toothpicks were used as negative controls. The inoculated plants were 169 maintained in a greenhouse at an average temperature of 35°C for 30 days, under natural 170 171 daylight conditions. Thirty days after inoculation, the aggressiveness of the isolates was assessed as disease severity based on the modified version of the rating scale described 172 by Ambrósio et al. (2015), where, 0 = symptomless, 1 = less than 3 % of shoot tissues 173 174 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. Seven 175 small fragments (0.2–0.5 cm) of necrotic lesions from each symptomatic plant were cut 176 and placed on PDAS in an attempt to recover the inoculated fungi and complete Koch's 177 postulates. The experiment was arranged in a completely randomized design with five 178 replicates per treatment (isolate) and one plant per replicate. The experiment was 179 conducted twice. A preliminary ANOVA was performed to determine whether there were 180 significant differences between the two repetitions of the experiment and whether the data 181 182 could be combined. Disease incidence was determined as the total number of infected plants from each Macrophomina species and expressed as percentage. Disease severity 183 results were analyzed with the nonparametric Kruskal–Wallis test at the probability level 184 of 5% using the software Assistat, version 7.7 (Silva and Azevedo 2016). 185

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187 3 RESULTS
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188

# **3.1 Effect of temperature on mycelial growth of** *Macrophomina pseudophaseolina*

190

The mycelial growth rates (MGR) significantly differed ( $p \le 0.05$ ) among the 62 191 *M. pseudophaseolina* isolates in PDA, and are shown in Table 2. The cubic polynomial 192 regression ( $y=a+bx+cx^2+dx^3$ ) selected to describe the mycelial growth at different 193 temperatures fitted the data with  $R^2 > 0.90$  for all the isolates. The optimum growth 194 temperatures for all isolates ranged between 26.4 (CMM4765) and 38.1°C (CMM4820) 195 196 (Table 2). Using the Scott-Knott univariate grouping test, a high variability of mycelial growth rate was observed between isolates, three groups of isolates at 25°C, five groups 197 at 30, 35 and 40°C were revealed (Table 2). At 25°C, the mean MGR was 7.5 mm d<sup>-1</sup> and 198 the values of this variable ranged from 4.3 (CMM4810) to 10.7 mm d<sup>-1</sup> (CMM4779). 199

200	MGR values of $\ge 8 \text{ mm d}^{-1}$ were observed in 37.1% of the isolates incubated at 25°C. At
201	30°C, the mean MGR was 9.1 mm $d^{-1}$ and the values ranged between 5.2 (CMM4814)
202	and 13.1 mm d <sup>-1</sup> (CMM4771). MGR $\ge$ 8 mm d <sup>-1</sup> were observed in 83.9% of the isolates.
203	At 35°C, the mean MGR was 10.1 mm d <sup>-1</sup> , the values ranged between 2.3 (CMM4814)
204	and 20.5 mm d <sup>-1</sup> (CMM4795 and CMM4797), and 59.7% of the isolates showed MGR $\geq$
205	8 mm/day. At 40°C, the mean MGR was 5.1 mm d <sup>-1</sup> , the values ranged between 1.0
206	(CMM4823) and 18.7 mm d <sup>-1</sup> (CMM4820), and 30.6% of the isolates showed MGR $\ge 8$
207	mm/day. None of the isolates were able to grow on PDA at 45°C.

# 209 **3.2 Effect of salinity on mycelial growth of** *M. pseudophaseolina*

210

The scatter plot of the effect of salinity on MGR of the 62 *M. pseudophaseolina* isolates is shown in Figure 1. The NaCl concentrations adjusted means of the all isolates were subjected to a regression analysis, and showed significant positive correlations (p <0.01). As the NaCl concentration was increased, MGR of all *M. pseudophaseolina* isolates were reduced *in vitro* on PDA.

Statistically significant effects of the 62 M. pseudophaseolina isolates for each 216 NaCl concentration on MGR were observed (p < 0.001). Using the Scott-Knott univariate 217 grouping test, four groups of isolates at 0, 250, and 750 mM, two groups of isolates at 218 500 mM, and six groups at 1000 mM, were revealed (Table 2). At 0 mM, the mean GR 219 was 8.7 mm d<sup>-1</sup> and the values of this variable ranged from 5.2 (CMM4814) to 11.6 mm 220 d<sup>-1</sup> (CMM4821). At 250 mM, the mean MGR was 2.7 mm d<sup>-1</sup> and the values ranged 221 between 1.0 (CMM4775) and 4.5 mm d<sup>-1</sup> (CMM4812). This concentration showed 69.0% 222 223 reduction in the MGR in relation to the concentration of 0 mM. At 500 mM, the mean MGR was 1.0 mm d<sup>-1</sup>, the values ranged between 0.6 (CMM4816) and 1.8 mm d<sup>-1</sup> 224

(CMM4773), and 88.5% of reduction of the MGR. At 750 mM, the mean MGR was 0.7 mm d<sup>-1</sup>, the values ranged between 0.3 (CMM4789 and CMM4797) and 1.1 mm d<sup>-1</sup> (CMM4768 and CMM4782), and showed 91.9% of reduction in the MGR in relation of the concentration of 0 mM. At 1000 mM, the mean MGR was 0.4 mm d<sup>-1</sup>, the values ranged between 0.1 (CMM4765 and CMM4797) and 0.9 mm d<sup>-1</sup> (CMM4782), and showed 95.4% of reduction of the MGR.

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## 232 **3.3** Sensitivity of *M. pseudophaseolina* to fungicide carbendazim

233

The effects of different concentrations of carbendazim on mycelial radial growth of the 62 *M. pseudophaseolina* isolates are shown in Table 3. By measuring the *M. pseudophaseolina* colony diameter in each treatment, regression equations for log-probit were adjusted and the EC<sub>50</sub> values were calculated. The mean EC<sub>50</sub> was 0.057 mg L<sup>-1</sup> a.i. and the values of this variable ranged from 0.013 to 0.089 mg L<sup>-1</sup> a.i. of carbendazim.

# 240 3.4 Pathogenicity and aggressiveness on melon and watermelon seedlings

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242 The inoculation with *M. pseudophaseolina* isolates caused significant statistical effect on disease severity in melon seedlings ( $\chi^2 = 142$ ; p < 0.05) and watermelon 243 seedlings ( $\chi^2 = 120.4$ ; p < 0.05) (Table 3). Per cent recovery of the inoculated isolates 244 from the necrotic tissues of symptomatic plants was higher than 90%, and the reisolates 245 246 were confirmed to be the same previously inoculated. No isolates were obtained from the negative controls for each crop. In melon, the highest disease severity mean (1.8) was 247 produced by isolate CMM-4826, while in watermelon isolates CMM-4808 and CMM-248 4823 produced the highest disease severity mean (0.6). Thus, these were considered to be 249

the most aggressive isolates for each cucurbit species. The lowest disease severity mean 250 (0.2) was produced by isolates CMM4807, CMM4808 and CMM4823, while in 251 watermelon isolates CMM4790, CMM4802, CMM4814, CMM4815 and CMM4822 252 presented the highest disease severity mean (0.2), thus, they were considered the least 253 aggressive isolates for each cucurbit species. In melon, the isolates CMM4771, 254 CMM4776, CMM4777, CMM4778, CMM4779, CMM4780, CMM4788, CMM4798, 255 CMM4805, CMM4806 and CMM4824 presented intermediate aggressiveness, with 256 257 disease severity mean values ranging from 0.4 to 1.2, the other isolates were not pathogenic (Table 3). In watermelon, the isolate CMM4801 (0.4) presented intermediate 258 aggressiveness, the other were not pathogenic to this crop (Table 3). In general, the M. 259 pseudophaseolina isolates studied, regardless of the host of origin, were more aggressive 260 to melon (0.21) than to watermelon (0.04), considering the average of all isolates for each 261 262 crop species (Table 3).

263

#### 264 4 DISCUSSION

265

This research, conducted with 62 isolates of *M. pseudophaseolina*, revealed variability regarding mycelial growth at different temperatures and salinity concentrations, their sensitivity to carbendazim, and their aggressiveness to melon and watermelon seedlings.

The optimum temperature for mycelial growth of *M. pseudophaseolina* isolates varied between 26.4 and 38.1°C. These values are in contrast with the results obtained by Sarr et al. (2014), who showed optimal growth range between 30 and 36°C for *M. pseudophaseolina* isolates obtained from *A. esculentus*, *A. hypogaea*, *H. sabdariffa* and *V. unguiculata*, in Senegal. The optimum temperature for mycelial growth of *M.*  *pseudophaseolina* found in our study fits with the typical soil temperatures at planting
and the average soil temperatures, 22.6°C (28.2 °C) 33.8°C, during watermelon and melon
reproductive growth stages at RN and CE states, Northeastern Brazil (Castellane and
Cortez 1995; Figueirêdo et al. 2017). Therefore, our results suggest that Brazilian *M. pseudophaseolina* isolates may be adapted to root infection in the early growth stages and
during subsequent plant development under heat and drought stress conditions.

Regarding salinity, our study showed that NaCl reduced the *in vitro* growth of all 281 282 M. pseudophaseolina isolates. The NaCl concentration of 250 mM caused more than a 50% reduction of *M. pseudophaseolina* mycelial growth. Similar results were reported in 283 284 M. phaseolina by Cervantes-García et al. (2003) and Tijerina-Ramírez et al. (2014). These authors indicated that this fungus spent more energy to obtain water molecules as the 285 NaCl concentration increased, reducing the mycelial growth of the fungus under in vitro 286 287 conditions. Similarly, in our case increasing the NaCl concentration reduced mycelial 288 growth of *M. pseudophaseolina in vitro*, probably because NaCl trapped water molecules 289 available in the BDA medium for the fungus; being the osmotic potential lower in the 290 fungal cell compared to the conditions of the BDA medium.

The *M. pseudophaseolina* isolates were highly sensitive to the fungicide 291 carbendazim, exhibiting EC<sub>50</sub> values ranging from 0.013 to 0.089 mg  $L^{-1}$  a.i. The efficacy 292 of the fungicide carbendazim at low concentrations (<1 mg L<sup>-1</sup>) in vitro was already 293 294 proved by Tonin et al. (2013) for the species M. phaseolina. The EC<sub>50</sub> is specific and constant for a particular chemical agent and to a particular pathogen, and a low EC<sub>50</sub> value 295 296 indicates a high fungicidal action or fungicidal power (Reis et al. 2010). These results indicate that the use of carbendazim is a measure that could be suggested for the 297 298 management of charcoal stem rot (Ndiaye et al. 2015). In Brazil, carbendazim is currently authorized for some seed treatments (Agrofit 2020). 299

300 Disease severity values on melon and watermelon obtained in this study were useful for discriminating among isolates of *M. pseudophaseolina* and confirmed that this 301 302 species is more aggressive in melon than in watermelon. This knowledge should be considered if breeding programs for resistance to charcoal rot of melon and watermelon 303 are going to incorporate this new species. The M. pseudophaseolina isolates were re-304 isolated from all symptomatic plants, fulfilling Koch's postulates. Macrophomina 305 pseudophaseolina was previously reported as aggressive in cowpea varieties in Senegal 306 307 (Ndiaye et al. 2015). The pathogenicity in melon and watermelon of *M. pseudophaseolina* was already determined by Negreiros et al. (2019) using a more reduced set of 10 isolates. 308 309 These authors also found that disease severity caused by *M. pseudophaseolina* was higher in melon than in watermelon. 310

311 Information about adaptability components of *M. pseudophaseolina* obtained in 312 this study could be incorporated on breeding programs for melon and watermelon crops.

313

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#### 319 **REFERENCES**

Agrofit. (2020) Sistemas de agrotóxicos fitossanitários. Internet Resource:
http://agrofit.agricultura.gov.br/agrofit\_cons/principal\_agrofit\_cons (verified Mar
20, 2020).

- 323 Ambrósio MMQ, Dantas ACA, Martínez-Perez E, Medeiros AC, Nunes GHS, Picó MB.
- 324 (2015) Screening a variable germplasm collection of *Cucumis melo* L. for seedling
   325 resistance to *Macrophomina phaseolina*. Euphytica, 206: 287-300
- Antonovics J, Alexander HM. (1989) The concept of fitness in plant fungal pathogen
  systems. In: Leonard KJ, Fry WE. (Eds.) Plant disease epidemiology. New York,
- 328 NY, USA, McGraw-Hill. pp 185-214.
- 329 Brito ACQ, Mello JF, Michereff SJ, Souza-Motta CM, Machado AR. (2019) First report
- of *Macrophomina pseudophaseolina* causing stem dry rot in cassava in Brazil. Plant
- **331** Pathol. J. 101: 1245-1245
- Castellane PD, Cortez GEP. (1995) A cultura da melancia (p. 64). Jaboticabal, SP, Brasil,
   FUNEP, Universidade Estadual Paulista.
- Cervantes-Garcia DC, Ramirez JSP, Simpson J, Mayek-Perez J. (2003) Osmotic
  potential effects on in vitro growth, morphology and pathogenicity of

336 *Macrophomina phaseolina*. J Phytopathol. 151: 456-462

337 Dhingra OD, Sinclair JB. (1978) Biology and pathology of *Macrophomina phaseolina*.

1st ed., Viçosa, MG, Brazil, Imprensa Universitária - UPV. 166p.

- 339 Farr DF, Rossman AY. (2019) Fungal Databases, U.S. National Fungus Collections,
- ARS, USDA. Internet Resource: https://nt.ars-grin.gov/fungaldatabases/. (verified
  Aug 29, 2019).
- Ferreira DF. (2011) A computer statistical analysis system. Ciênc. Agrotec. 35: 10391042.
- Figueiredo MCB, Gondim RS, Aragão FAS. (2017) Produção de melão e mudanças
  climáticas: sistemas conservacionistas de cultivo para redução das pegadas de
  carbono e hídrica (p. 302). Brasília, Brasil: Empresa Brasileira de Pesquisa
  Agropecuária, EMBRAPA-CNPAT.

348	Gonçalves CR, Alfenas AC, Mafia RG. (2016) Armazenamento de Microrganismos em
349	Cultura com Ênfase em Fungos Fitopatogênicos. In Alfenas AC, Mafia RG. (Eds.),
350	Métodos em fitopatologia, 2ª ed. Viçosa, MG, Brasil, pp 93-105.
351	Gupta GK, Sharma SK, Ramteke, R. (2012) Biology, epidemiology and management of
352	the pathogenic fungus Macrophomina phaseolina (Tassi) Goid with special reference
353	to charcoal rot of soybean (Glycine max (L.) Merrill). J Phytopathol 160: 167-180
354	Lannou C. (2012) Variation and selection of quantitative traits in plant pathogens. Annu
355	Rev Phytopathol 50: 319-338
356	Machado AR, Pinho DB, Dartanhã JS, Gomes AAM, Pereira OL. (2019) Bayesian
357	analyses of five gene regions reveal a new phylogenetic species of Macrophomina
358	associated with charcoal rot on oilseed crops in Brazil. Eur J Plant Pathol 153: 89-
359	100
360	Mastan A, Bharadwaj RKB, Kushwaha RK, Vivek Babu CS. (2019) Functional Fungal
361	Endophytes in Coleus forskohlii Regulate Labdane Diterpene Biosynthesis for
362	Elevated Forskolin Accumulation in Roots. Microb Ecol. 78: 914-926
363	Mayek-Pérez N, López-Castañeda C, Acosta-Gallegos CJA. (1997) Variación en
364	características culturales in vitro de aislamientos de Macrophomina phaseolina y su
365	virulencia en frijol. Agrociencia 31, 187-195
366	Medeiros AC, Melo DRM, Ambrósio MMQ, Nunes GHS, Costa JM. (2015) Methods of
367	inoculation of Rhizoctonia solani and Macrophomina phaseolina in melon (Cucumis
368	melo). Summa Phytopathol. 41: 281–286

- 369 Ndiaye M, Sarr P, Cisse N, Ndoye I. (2015) Is the recently described Macrophomina
- 370 *pseudophaseolina* pathogenically different from *Macrophomina phaseolina*?. Afr J
- 371 Microbiol Res. 9: 2232-2238

372	Negreiros AMP,	, Sales Jú	nior R, León M	l, Melc	NJA, Miche	reff SJ, Ambró	s10 MMQ,
373	Armengol .	J. (2019)	Identification	and r	athogenicity	of Macrophon	mina species

- 374 collected from weeds in melon fields in Northeastern Brazil. J Phytopathol. 167: 326375 337
- Reis EM, Reis AC, Forcelini CA. (2010) Manual de fungicidas: guia para o controle
  químico de doenças de plantas. Passo Fundo, Brasil: UPF editora, Universidade de
  Passo Fundo
- 379 Sarr MP, Ndiaye M, Groenewald JZ, Crous PW. (2014) Genetic diversity in
   380 *Macrophomina phaseolina*, the causal agent of charcoal rot. Phytopathol Mediterr.
   381 53: 163-173
- 382 Silva FAZ, Azevedo CAV. (2016) The Assistat Software Version 7.7 and its use in the
  analysis of experimental data. Afr J Agric Res. 11: 3733-3740
- 384 Systat Software, Inc. (2002). TableCurve 2D, version 5.01. Internet Resource:
  385 https://systatsoftware.com/. (verified Mar 29, 2020)
- 386 Tijerina-Ramírez N, Lira-Méndez K, Moreno-Medina VR, González-Prieto JM, Mayek-
- 387Pérez N. (2014) Osmotic stress effect on *in vitro* growth, pathogenicity and osmolyte

388 production in *Macrophomina phaseolina*. Rev Mex Micol. 39: 31-39

- 389 Tonin RFB, Avozani A, Danelli ALD, Reis EM, Zoldan SM, Garcés-Fiallos FR. (2013)
- *In vitro* mycelial sensitivity of *Macrophomina phaseolina* to fungicides. Pesqui
  Agropecu Trop. 43: 460-466
- Wrather JA, Anderson TR, Arsyad DM, Tan Y, Ploper LD, Porta-Puglia, ... Yorinori JT.
- 393 (2001) Soybean disease loss estimates for the top 10 soybean producing countries in
- 394 1998. Can J of Plant Pathol 23: 115–221
- Zhan J, McDonald BA. (2013) Experimental measures of pathogen competition and
- relative fitness. Annu Rev Phytopathol. 51:131-153

- 397 Zhao L, Cai J, He W, Zhang Y. (2019) Macrophomina vaccinii sp. nov. causing
- 398blueberry stem blight in China. MycoKeys 55: 1-14

400	Tables	Captions
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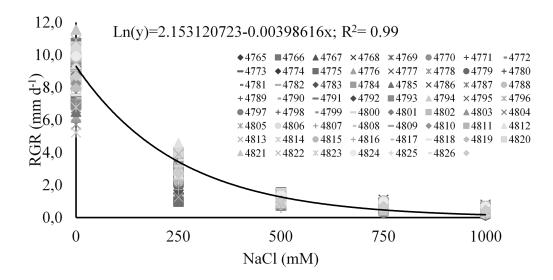
402	<b>TABLE 1</b>	List of Macro	phomina	pseudo	phaseolina	isolates	used in	this stu	dy

- **TABLE 2** Optimum temperature and mean mycelial growth rate at 25, 30, 35, and 40 °C,
- and mycelial growth rate at 0, 250, 500, 750, and 1000 mM of NaCl, of *Macrophomina*
- *pseudophaseolina* isolates from northeastern Brazil

- **TABLE 3** Regression equation and 50% inhibitory concentration of mycelium growth
- 409 (EC<sub>50</sub>) for log Probit analysis by fungicide carbendazim, and disease severity and
- 410 incidence induced to melon and watermelon seedlings by 62 isolates of *Macrophomina*
- *pseudophaseolina* from northeastern Brazil

413	Figure	Captions
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FIGURE 1 Scatter plot of average relative growth rate (RGR) of 62 isolates of *Macrophomina pseudophaseolina* on potato-dextrose-agar adjusted to different NaCl
concentrations



**FIGURE 1** Scatter plot of average relative growth rate (RGR) of 62 isolates of *Macrophomina pseudophaseolina* on potato-dextrose-agar adjusted to different NaCl concentrations

Strain number (CMM) <sup>a</sup>	Host	Location <sup>b</sup>
4765, 4766, 4767, 4768	Trianthema portulacastrum	Brazil, CE, Icapuí
4769, 4770, 4771, 4772, 4773,	Trianthema portulacastrum	Brazil, RN, Assú
4774, 4775, 4776, 4777, 4778		
4779, 4780, 4781, 4782, 4783,	Trianthema portulacastrum	Brazil, RN, Mossoró
4784, 4785, 4786, 4787, 4788		
4789, 4790, 4791, 4792, 4793,	Boerhavia diffusa	Brazil, RN, Assú
4794, 4795, 4796, 4797, 4798,		
4799, 4800, 4891, 4892, 4893,		
4894, 4810, 4811, 4812, 4813		
4814, 4815, 4816, 4817, 4818,	Boerhavia diffusa	Brazil, RN, Mossoró
4819 4820, 4821, 4822, 4823,		
4824, 4825, 4826		

**TABLE 1** List of Macrophomina pseudophaseolina isolates used in this study

<sup>a</sup>CMM - Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" of the Universidade Federal Rural de Pernambuco (Recife, PE, Brazil).

<sup>b</sup>Ceará state = CE and Rio Grande do Norte state = RN.

428

430 TABLE 2 Optimum temperature and mean mycelial growth rate at 25, 30, 35, and 40 °C, and mycelial growth rate at 0, 250, 500, 750, and 1000

Isolates	Optimum		Tempe	rature				Salinity		
(CMM) <sup>a</sup>	temperature	Мус	elial Growth R	ate (mm d <sup>-1</sup> ) $\pm$	SE <sup>b</sup>		Mycelial Gro	owth Rate (mi	$m d^{-1}$ ) $\pm SE^{b}$	
	(°C)	25°C	30°C	35°C	40°C	0	250	500	750	1000
4765	26.4	9.5 ± 0.2 a	$9.0 \pm 0.4$ c	$4.5 \pm 0.1  e$	$2.2 \pm 0.1 \ e$	$10.5 \pm 0.2$ a	$1.7 \pm 0.1 \text{ d}$	$0.9\pm0.1~\text{b}$	$0.7 \pm 0.1$ c	$0.1 \pm 0.1$ 1
4787	26.8	$8.5\pm0.1$ a	$8.6\pm0.3~d$	$4.3 \pm 0.1  e$	$2.1 \pm 0.1  e$	$8.5\pm0.1\ c$	$2.5\pm0.2\ c$	$1.2\pm0.2$ a	$0.6\pm0.1~\text{c}$	$0.3 \pm 0.1$ c
4777	26.8	$6.7\pm0.1~\text{b}$	$6.9\pm0.3~e$	$3.4 \pm 0.1$ e	$1.7 \pm 0.1  e$	$6.7\pm0.1~d$	$3.1\pm0.2\ b$	$1.3 \pm 0.1$ a	$0.7\pm0.1~\text{c}$	$0.4 \pm 0.1$ c
4769	26.8	$8.2\pm0.7$ a	$8.4\pm0.7\;d$	$4.2\pm0.2$ e	$2.1\pm0.1$ e	$8.2\pm0.7\ c$	$3.3\pm0.5\ b$	$1.1\pm0.1\;b$	$0.7\pm0.1$ a	$0.4 \pm 0.1$ c
4773	26.8	$8.5\pm0.6$ a	$8.7\pm0.9\;d$	$4.3 \pm 0.2$ e	$2.2\pm0.2$ e	$8.5\pm0.6\ c$	$4.1 \pm 0.2$ a	$1.8\pm0.1$ a	$0.8\pm0.1\;b$	$0.3 \pm 0.1$ e
4784	26.8	$8.9\pm0.3~a$	$9.1\pm0.6\ c$	$4.6 \pm 0.1$ e	$2.3\pm0.1~\text{e}$	$8.9\pm0.3\ b$	$2.5\pm0.4\;c$	$1.0\pm0.1\ b$	$0.8\pm0.1\;b$	$0.5 \pm 0.1$ o
4766	26.8	$9.3\pm0.4\ a$	$9.5\pm1.9\ c$	$4.7\pm0.5~e$	$2.4\pm0.2~\text{e}$	$9.3\pm0.4\ b$	$2.5\pm0.4\;c$	$0.9\pm0.1\;b$	$0.7\pm0.1~\text{c}$	$0.5 \pm 0.1$ o
4779	27.1	$10.7 \pm 1.0$ a	$11.3 \pm 1.7 \text{ b}$	$5.6 \pm 0.4$ e	$2.8\pm0.2~\text{e}$	$10.8 \pm 1.0$ a	$2.0\pm0.2\;d$	$1.2\pm0.1\ b$	$0.9\pm0.2$ a	$0.3 \pm 0.1$ c
4780	27.1	$8.3\pm0.2\ a$	$8.8\pm0.7\;d$	$4.4\pm0.2~e$	$2.2\pm0.1~\text{e}$	$8.4\pm0.2\ c$	$2.7\pm0.3\ c$	$1.1\pm0.1\;b$	$0.8\pm0.1~a$	$0.5 \pm 0.1$ c
4781	27.3	$8.3\pm0.3~a$	$9.0\pm0.5\ c$	$4.5\pm0.1$ e	$2.2\pm0.1$ e	$8.3\pm0.3\ c$	$2.2\pm0.1\ c$	$1.0\pm0.1\;b$	$0.7\pm0.3$ a	$0.4 \pm 0.1$ o
4783	27.3	$9.0\pm0.5$ a	$9.7\pm0.9\ c$	$4.8 \pm 0.2  e$	$2.4 \pm 0.1$ e	$9.0\pm0.5\ b$	$1.5\pm0.2\;d$	$0.9\pm0.1\;b$	$0.5\pm0.1\ d$	$0.2 \pm 0.1$
4786	27.6	$8.2\pm0.1$ a	$9.1\pm0.8\ c$	$4.6 \pm 0.2$ e	$2.3\pm0.1 \text{ e}$	$8.3\pm0.1\ c$	$2.5 \pm 0.2$ c	$0.9\pm0.1\ b$	$0.9\pm0.1\;b$	$0.3 \pm 0.1$

431 mM of NaCl, of *Macrophomina pseudophaseolina* isolates from northeastern Brazil

4772	27.6	$9.1 \pm 1.0 \text{ a}$	$10.1 \pm 0.8$ c	$5.1 \pm 0.2$ e	$2.5\pm0.2~\text{e}$	$9.1\pm1.0~\text{b}$	$1.4\pm0.2~d$	$1.0\pm0.1\ b$	$0.9\pm0.1\ b$	$0.5\pm0.1\ c$
4785	27.9	$6.5\pm0.7\ b$	$7.6 \pm 3.4$ e	$3.8\pm0.8\ e$	$1.9\pm0.4~\text{e}$	$6.5\pm0.7\;d$	$1.2\pm0.1\ d$	$0.8\pm0.1\ b$	$0.7\pm0.1~\text{c}$	$0.4\pm0.1\ d$
4774	28.0	$8.0\pm0.3~a$	$9.3\pm0.3\;d$	$4.7\pm0.1~\text{e}$	$2.3\pm0.1 \text{ e}$	$8.0\pm0.3\ c$	$1.5\pm0.2\;d$	$1.0\pm0.1\ b$	$0.9\pm0.1\ b$	$0.4\pm0.1\ d$
4767	28.0	$8.9\pm1.4~a$	$10.4\pm0.8\ c$	$5.2\pm0.2~\text{e}$	$2.6\pm0.1\ e$	$8.9\pm1.4\ b$	$3.1\pm0.3\ b$	$1.3 \pm 0.1$ a	$0.7\pm0.1~\text{c}$	$0.6\pm0.1\;b$
4776	28.1	$6.2\pm0.1~\text{c}$	$7.3\pm0.7\;e$	$3.7\pm0.2~\text{e}$	$1.8\pm0.2~\text{e}$	$6.2\pm0.2\;d$	$1.7\pm0.2\ d$	$1.1 \pm 0.1 \ a$	$0.9\pm0.1\ b$	$0.5\pm0.1\ c$
4770	28.2	$6.8\pm0.1\ b$	$8.2\pm0.5\;d$	$4.1\pm0.1~\text{e}$	$2.1\pm0.1~\text{e}$	$6.8\pm0.1\ d$	$2.6\pm0.5\ c$	$1.0\pm0.1\ b$	$0.8\pm0.1~a$	$0.5\pm0.1\ c$
4768	28.2	$8.4\pm0.7\ a$	$10.2\pm0.9~\text{c}$	$5.1\pm0.2~\text{e}$	$2.5\pm0.1 \ e$	$8.4\pm0.7\ c$	$2.7\pm0.3\ c$	$1.3 \pm 0.1$ a	$1.1 \pm 0.1 \ a$	$0.8\pm0.1\ a$
4788	28.2	$8.9\pm0.4\ a$	$10.9\pm2.0\ b$	$5.4\pm0.5~\text{e}$	$2.7\pm0.2~\text{e}$	$8.9\pm0.4\ b$	$2.9\pm0.2\;b$	$1.4\pm0.2\ a$	$1.0 \pm 0.1$ a	$0.6\pm0.1\;b$
4771	28.5	$10.1\pm0.3~a$	$13.1\pm1.8\ a$	$6.5\pm0.4~e$	$3.2\pm0.2\ e$	$10.1\pm0.2~a$	$3.4\pm0.4\;b$	$0.9\pm0.1\ b$	$0.7\pm0.1~\text{c}$	$0.3\pm0.1\ d$
4778	28.7	$5.7\pm0.1\ c$	$7.7\pm0.7\;e$	$3.8\pm0.2~\text{e}$	$1.9\pm0.2~\text{e}$	$5.7\pm0.3\ d$	$2.3\pm0.3\ \text{c}$	$0.9\pm0.1\ b$	$0.8\pm0.1~\text{c}$	$0.3\pm0.1\ e$
4782	28.8	$7.9\pm0.8\ a$	$10.9\pm0.2\ b$	$5.4\pm0.1~\text{e}$	$2.7\pm0.1~\text{e}$	$7.9\pm0.8\ c$	$2.1\pm0.9\ c$	$1.2 \pm 0.3$ a	$1.1 \pm 0.1$ a	$0.9\pm0.1\ a$
4775	28.9	$7.2\pm0.3\ b$	$10.3\pm1.6\ c$	$5.1\pm0.4~\text{e}$	$2.6\pm0.4\ e$	$7.2\pm0.2\ d$	$1.0\pm0.1\ d$	$0.9\pm0.1\ b$	$0.9\pm0.1\ b$	$0.6\pm0.1\;b$
4823	29.8	$7.3\pm0.3\;b$	$6.8 \pm 1.0 \text{ e}$	$10.8\pm1.5~d$	$1.0 \pm 0.1 \text{ e}$	$6.8\pm0.5\;d$	$3.4\pm0.1\ b$	$0.9\pm0.1\ b$	$0.6\pm0.1\ d$	$0.4\pm0.1\ d$
4792	30.0	$7.3\pm0.6\;b$	$9.3\pm1.8\;c$	$10.2\pm0.9~d$	$1.9\pm0.1~\text{e}$	$9.3\pm0.9\ b$	$3.2\pm1.2\;b$	$0.9\pm0.1\ b$	$0.7\pm0.1~\text{c}$	$0.3\pm0.1\ e$
4796	30.2	$6.1 \pm 0.3$ c	$8.0\pm0.8\;d$	$8.8\pm1.9~e$	$2.2\pm0.1\ e$	$8.1\pm0.4\ c$	$2.8\pm0.4\ c$	$1.0\pm0.1\ b$	$0.8\pm0.1~\text{c}$	$0.5\pm0.1\ c$
4791	30.5	$7.7\pm0.1\ b$	$11.0\pm0.9~b$	$13.3 \pm 1.2$ c	$2.3\pm0.1 \text{ e}$	$11.0\pm0.5~a$	$3.2\pm0.2\;b$	$1.6 \pm 0.1 \ a$	$0.8\pm0.1~\text{c}$	$0.5\pm0.1\ c$
4794	30.5	$5.9 \pm 1.2$ c	$9.2\pm1.4\;c$	$10.7\pm1.8~d$	$2.0\pm0.2\ e$	$9.2\pm0.7\;b$	$3.4\pm0.2\;b$	$1.1 \pm 0.1 \; a$	$0.7\pm0.1~\text{c}$	$0.4\pm0.1\ d$
4824	30.5	$7.2\pm0.6\;b$	$9.9\pm0.3\ c$	$12.7\pm0.8~\text{c}$	$2.1\pm0.1~\text{e}$	$10.0\pm0.1\ b$	$2.7\pm0.1\ c$	$1.4\pm0.1~a$	$1.0\pm0.1\ b$	$0.6\pm0.1\;b$

4811	30.7	$6.9 \pm 0.1$ b	$9.9\pm0.5\ c$	$13.1 \pm 0.8$ c	$2.1 \pm 0.2$ e	$9.9\pm0.2\;b$	$4.0\pm0.2\ a$	$1.3 \pm 0.1 \text{ a}$	$0.8\pm0.1~\text{c}$	$0.7\pm0.1\;b$
4790	30.9	$6.9\pm0.3\ b$	$8.2\pm0.6\;d$	$10.5\pm0.1\ d$	$3.3\pm0.2\ e$	$8.3\pm0.3\ c$	$2.7\pm0.4\ c$	$0.9\pm0.1\ b$	$0.5\pm0.1\ d$	$0.3\pm0.1\;d$
4806	31.2	$7.4\pm0.2\ b$	$8.1\pm0.9\;d$	$15.0\pm1.1\ b$	$2.1\pm0.1~\text{e}$	$8.1\pm0.5~\text{c}$	$2.2\pm0.3\ c$	$0.8\pm0.1\ b$	$0.5\pm0.1\ d$	$0.2\pm0.1~\text{e}$
4789	31.3	$7.0\pm0.3\ b$	$8.5\pm0.6\;d$	$15.6\pm0.1\ b$	$2.1\pm0.2~\text{e}$	$8.5\pm0.3\ c$	$2.7\pm0.3\ c$	$0.8\pm0.1\ b$	$0.3\pm0.1 \ d$	$0.2\pm0.1~\text{e}$
4817	31.8	$6.4\pm0.5~\text{c}$	$9.8\pm1.6\;c$	$16.9\pm1.4\ b$	$3.2\pm0.3~\text{e}$	$9.9\pm0.8\ b$	$2.3\pm0.1\ c$	$1.4\pm0.1~a$	$0.8\pm0.1\ c$	$0.6\pm0.1\ c$
4807	31.8	$6.8\pm0.2\ b$	$10.0\pm0.7\ c$	$14.8\pm0.5~b$	$4.3\pm0.1\ d$	$10.0\pm0.3~b$	$3.0\pm0.2\;b$	$1.2\pm0.1$ a	$0.7\pm0.1\ c$	$0.3\pm0.1\ e$
4826	32.0	$8.2\pm0.3\ a$	$8.5\pm1.1~d$	$15.8\pm2.7\ b$	$3.8\pm0.5\ d$	$8.5\pm0.5~\text{c}$	$3.5\pm0.5\;b$	$0.9\pm0.1\ b$	$0.5\pm0.1\ d$	$0.5\pm0.1\ c$
4801	32.6	$7.4\pm0.1\ b$	$8.6\pm0.2\;d$	$9.7\pm0.2\ d$	$5.9\pm1.3~\text{d}$	$8.6\pm0.1~\text{c}$	$3.8\pm0.1\ a$	$1.5\pm0.1\ a$	$0.5\pm0.1\ d$	$0.3\pm0.1\ d$
4795	32.8	$6.3\pm0.7~\text{c}$	$9.1\pm0.9\;c$	$20.5\pm0.1\ a$	$4.2\pm0.4\ d$	$9.1\pm0.4\ b$	$2.5\pm0.2\ c$	$1.1\pm0.1~a$	$0.4\pm0.1\ d$	$0.2\pm0.1~\text{e}$
4797	32.9	$8.3\pm0.3\ a$	$9.6\pm0.7\ c$	$20.5\pm0.1\ a$	$4.9\pm0.7\;d$	$9.6\pm0.4\ b$	$2.1\pm0.1\ \text{c}$	$0.7\pm0.1\ b$	$0.3\pm0.1 \ d$	$0.1\pm0.1\ f$
4810	32.9	$4.3\pm1.4\ c$	$8.3\pm0.7\;d$	$17.1\pm1.8~b$	$4.2\pm0.6\;d$	$8.3\pm0.3\ c$	$2.2\pm0.2\ c$	$0.8\pm0.1\ b$	$0.5\pm0.1\;d$	$0.4\pm0.1\ d$
4814	33.1	$5.4\pm0.9\ c$	$5.2\pm0.4\ e$	$2.3\pm0.3~\text{e}$	$4.1\pm0.2\ d$	$5.2\pm0.2\;d$	$3.1\pm0.4\ b$	$0.8\pm0.1\ b$	$0.5\pm0.1\;d$	$0.3\pm0.1\ d$
4818	33.5	$7.4\pm0.1\ b$	$7.6 \pm 1.5$ e	$11.1\pm0.8~d$	$5.5\pm1.6\;d$	$7.6\pm0.8\ c$	$2.4\pm0.4\ c$	$0.8\pm0.1\ b$	$0.6\pm0.1~\text{c}$	$0.3\pm0.1\ e$
4821	34.1	$7.3 \pm 1.3$ b	$11.6\pm1.2\ b$	$12.5\pm0.5~\text{c}$	$9.5\pm2.4\;c$	$11.6\pm0.6~a$	$3.1\pm0.1\;b$	$1.2\pm0.1$ a	$0.5\pm0.1 \ d$	$0.3\pm0.1\ d$
4815	34.8	$7.5\pm0.3\ b$	$9.8\pm1.3\ \text{c}$	$19.9\pm0.6\ a$	$8.5\pm0.4\;c$	$9.8\pm0.6\ b$	$2.3\pm0.2\ c$	$1.7\pm0.9\;a$	$0.6\pm0.1\ c$	$0.4\pm0.1\ d$
4802	35.1	$5.9\pm0.7\ c$	$8.4\pm0.5\;d$	$14.9\pm0.9~b$	$9.4\pm1.3~\text{c}$	$8.4\pm0.3\ c$	$2.9\pm0.2\;b$	$1.2\pm0.1$ a	$0.7\pm0.1\ c$	$0.3\pm0.1\;d$
4805	35.1	$7.1\pm0.7\;b$	$9.6\pm0.6\ c$	$15.1\pm0.4\ b$	$8.9\pm0.4\ c$	$9.6\pm0.3\ b$	$3.6\pm0.1\ a$	$0.9\pm0.1\ b$	$0.7\pm0.1\ c$	$0.5\pm0.1\ c$
4825	35.3	$7.2\pm1.2~\text{b}$	$9.0\pm0.4\ c$	$12.1\pm0.4~\text{c}$	$8.5\pm0.6\ c$	$9.1\pm0.2\ b$	$2.3\pm0.2\ c$	$0.7\pm0.1\ b$	$0.6\pm0.1~\text{c}$	$0.5\pm0.1\ c$

4922	25.4	75+021	10.0 + 2.2.1	145+051	10.5 + 1.2.1	10.0 + 1.2	20102	10+011	0.5 + 0.1.1	0.4 + 0.1.1
4822	35.4	$7.5\pm0.3~\text{b}$	$10.9\pm2.3~\mathrm{b}$	$14.5\pm0.5~b$	$10.5\pm1.2~b$	$10.9 \pm 1.2$ a	$3.9\pm0.3$ a	$1.0 \pm 0.1 \text{ b}$	$0.5\pm0.1$ d	$0.4 \pm 0.1 \ d$
4798	35.6	$7.7\pm0.2\ b$	$10.1\pm0.4\ c$	$15.7\pm0.4\ b$	$10.1 \pm 1.4$ c	$10.2\pm0.2~\text{a}$	$3.4\pm0.1\;b$	$1.1\pm0.1~\text{a}$	$0.6\pm0.1\ d$	$0.3\pm0.1\;d$
4819	35.7	$5.4\pm0.9\ c$	$8.0\pm0.4\;d$	$9.6\pm1.8\;d$	$8.2\pm2.1~\text{c}$	$8.0\pm0.2~\text{c}$	$2.8\pm0.2\ c$	$0.9\pm0.1\ b$	$0.8\pm0.1\ c$	$0.5\pm0.1\ c$
4804	36.0	$5.3 \pm 1.6$ c	$9.5\pm1.0\;\text{c}$	$19.1\pm1.4~a$	$12.3\pm0.7~\text{b}$	$9.6\pm0.5~\text{b}$	$3.2\pm0.1\ b$	$0.9\pm0.1\ b$	$0.5\pm0.1\ d$	$0.5\pm0.1\ c$
4813	36.0	$6.9\pm0.2\;\text{b}$	$9.6\pm1.0\;\text{c}$	$10.7\pm0.8~d$	$10.1\pm0.3~\text{c}$	$9.7\pm0.5\;b$	$1.2\pm0.2\ d$	$1.1\pm0.1~\text{a}$	$0.5\pm0.1\ d$	$0.4\pm0.1\ d$
4803	36.1	$9.3\pm0.8\ a$	$9.7\pm1.0\;\text{c}$	$15.9\pm0.8~\text{b}$	$11.8\pm1.0\text{ b}$	$9.7\pm0.5\;b$	$3.0\pm0.2\;b$	$0.9\pm0.1\ b$	$0.4\pm0.1\ d$	$0.4\pm0.1\ d$
4816	36.4	$7.3\pm0.5\;b$	$7.3\pm0.4\;e$	$11.3\pm0.3~d$	$8.0\pm0.3\ c$	$7.3\pm0.2 \ d$	$1.7\pm0.1\ d$	$0.6\pm0.1\ b$	$0.4\pm0.1\ d$	$0.2\pm0.1~\text{e}$
4808	36.4	$7.5\pm0.2\;b$	$7.4\pm2.5~e$	$13.0\pm0.8\ c$	$8.35\pm0.2\ c$	$7.4\pm1.2 \text{ d}$	$3.7\pm0.1\ a$	$0.8\pm0.1\ b$	$0.6\pm0.1\ c$	$0.4\pm0.1\ d$
4799	36.5	$7.7\pm0.3\ b$	$8.7\pm0.5\;d$	$14.1\pm0.5~b$	$10.2\pm0.4~\text{c}$	$8.7\pm0.3\;b$	$2.4\pm0.2\ c$	$1.1\pm0.1~\text{a}$	$0.8\pm0.1\ c$	$0.6\pm0.1\;b$
4812	36.7	$7.9\pm0.5\ a$	$9.5\pm1.4\;c$	$15.8\pm0.5~\text{b}$	$11.7\pm0.7~b$	$9.5\pm0.7\ b$	$4.5\pm0.1\ a$	$1.2\pm0.1\ a$	$0.7\pm0.1\ c$	$0.4\pm0.1\ d$
4809	36.9	$8.0\pm0.5\ a$	$9.0\pm0.5\ c$	$14.8\pm1.9~\text{b}$	$11.7\pm1.2~\text{b}$	$9.1\pm0.2\;b$	$3.0\pm0.1\;b$	$1.0\pm0.1\ b$	$0.7\pm0.1\ c$	$0.4\pm0.1\;d$
4800	37.1	$8.1\pm0.3\ a$	$9.7\pm0.5\ c$	$13.5\pm0.6\ c$	$12.5\pm2.1~b$	$9.7\pm0.3\;b$	$3.2\pm0.1\ b$	$1.2\pm0.1\ a$	$0.7\pm0.1\ c$	$0.5\pm0.1\ c$
4793	37.3	$6.6\pm0.7\;b$	$6.9 \pm 1.4$ e	$9.9\pm2.1\ d$	$9.3\pm0.7\ c$	$6.9\pm0.7\;d$	$2.5\pm0.7\ c$	$0.7\pm0.1\ b$	$0.6\pm0.1\ c$	$0.6\pm0.1\ c$
4820	38.1	$8.7\pm0.2\ a$	$10.5\pm0.6\ b$	$14.9\pm1.2~\text{b}$	$18.7\pm0.6\;a$	$10.5\pm0.3\ a$	$3.9\pm1.0\;a$	$0.9\pm0.1\ b$	$0.7\pm0.1\ c$	$0.5\pm0.1\ c$
Mean	31.4	7.5	9.1	10.1	5.1	8.7	2.7	1.0	0.7	0.4
CV (%)		16.7	12.3	17.7	27.9	12.7	25.1	15.1	20.2	23.6

432 <sup>a</sup> CMM - Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" of the Universidade Federal Rural de Pernambuco (Recife, PE, Brazil).

- 433 <sup>b</sup> Values are the mean of 10 measurements. SE = Standard Error of the Mean. Values with the same letter within a column (± standard error) are not significantly different
- 434 according to Scott-Knott test at 5% probability.

TABLE 3 Regression equation and 50% inhibitory concentration of mycelium growth (EC<sub>50</sub>) for log Probit analysis by fungicide carbendazim,
and disease severity and incidence induced to melon and watermelon seedlings by 62 isolates of *Macrophomina pseudophaseolina* from
northeastern Brazil

	Fungicide Carbe		Melon	1	Watermelon			
Isolates	Regression equation <sup>b</sup>	$EC_{50}^{c}$	Disease Disease		Dise	Disease		
(CMM) <sup>a</sup>		(mg L <sup>-1</sup> a.i.)	Sev	Severity		Severity		Incidence
			Rank	Mean <sup>e</sup>	(%)	Rank	Mean <sup>e</sup>	(%)
4792	y = 15.295x +	0.012	120.5	0.0	0.0	149.0	0.0	0.0
4782	78.772	0.013	139.5	0.0	0.0	149.0	0.0	0.0
	y = 15.769x +	0.010	120.5	0.0	0.0	1 40 0	0.0	0.0
4791	77.120	0.019	139.5	0.0	0.0	149.0	0.0	0.0
4000	y = 17.663x +	0.010	120.5	0.0	0.0	1 40 0	0.0	0.0
4800	74.048	0.019	139.5	0.0	0.0	149.0	0.0	0.0
4022	y = 16.707x +	0.025	120.5	0.0	0.0	100.0	0.2	20.0
4822	76.890	0.025	139.5	0.0	0.0	180.0	0.2	20.0

4821	y = 16.782x + 76.666	0.026	139.5	0.0	0.0	149.0	0.0	0.0
4768	y = 16.856x + 76.142	0.028	139.5	0.0	0.0	149.0	0.0	0.0
4798	y = 16.463x + 75.469	0.028	198.9	0.4	40.0	149.0	0.0	0.0
4788	y = 16.703x + 75.193	0.031	204.7	1.2	40.0	149.0	0.0	0.0
4815	y = 16.591x + 75.097	0.031	139.5	0.0	0.0	180.0	0.2	20.0
4789	y = 16.791x + 74.410	0.035	139.5	0.0	0.0	149.0	0.0	0.0
4786	y = 17.119x + 74.711	0.036	139.5	0.0	0.0	149.0	0.0	0.0

4797	y = 16.868x + 74.251	0.036	139.5	0.0	0.0	149.0	0.0	0.0
4811	y = 17.601x + 74.967	0.038	139.5	0.0	0.0	149.0	0.0	0.0
4819	y = 17.830x + 74.995	0.040	139.5	0.0	0.0	149.0	0.0	0.0
4820	y = 18.029x +	0.041	139.5	0.0	0.0	149.0	0.0	0.0
4775	75.070 y = 17.733x +	0.044	139.5	0.0	0.0	149.0	0.0	0.0
4794	74.075 y = 17.846x +	0.044	139.5	0.0	0.0	149.0	0.0	0.0
	74.183 y = 18.061x +							
4779	74.247	0.045	172.2	0.6	20.0	149.0	0.0	0.0

4771	y = 18.984x + 75.438	0.046	172.6	0.8	20.0	149.0	0.0	0.0
4805	y = 18.025x + 73.738	0.048	198.9	0.4	40.0	149.0	0.0	0.0
4807	y = 17.823x + 73.119	0.050	169.2	0.2	20.0	149.0	0.0	0.0
4778	y = 17.398x +	0.052	171.6	0.4	20.0	149.0	0.0	0.0
4792	72.355 y = 17.881x +	0.053	139.5	0.0	0.0	149.0	0.0	0.0
	72.842 y = 17.601x +							
4774	72.262 y = 18.801x +	0.054	139.5	0.0	0.0	149.0	0.0	0.0
4813	73.778	0.054	139.5	0.0	0.0	149.0	0.0	0.0

4825	y = 18.410x +	0.054	139.5	0.0	0.0	149.0	0.0	0.0
	73.275 y = 18.817x +							
4817	73.752	0.055	139.5	0.0	0.0	149.0	0.0	0.0
4824	y = 18.623x +	0.055	228.6	0.6	60.0	149.0	0.0	0.0
	73.528 y = 18.640x +							
4812	73.385	0.056	139.5	0.0	0.0	149.0	0.0	0.0
4773	y = 18.425x +	0.057	139.5	0.0	0.0	149.0	0.0	0.0
	72.860 y = 17.983x +							
4783	72.368	0.057	139.5	0.0	0.0	149.0	0.0	0.0
4804	y = 18.448x +	0.057	139.5	0.0	0.0	149.0	0.0	0.0
	72.882							

4780	y = 18.743x + 72.889	0.060	203.7	0.8	40.0	149.0	0.0	0.0
4781	y = 19.078x + 73.188	0.061	139.5	0.0	0.0	149.0	0.0	0.0
4806	y = 19.018x + 72.780	0.063	173.3	1.0	20.0	149.0	0.0	0.0
4809	y = 18.371x +	0.064	139.5	0.0	0.0	149.0	0.0	0.0
4814	71.908 y = 17.592x +	0.064	139.5	0.0	0.0	180.0	0.2	20.0
4796	70.965 y = 18.652x +	0.065	139.5	0.0	0.0	149.0	0.0	0.0
	72.099 y = 18.475x +							
4793	71.504	0.069	139.5	0.0	0.0	149.0	0.0	0.0

4799	y = 18.771x + 71.741	0.069	139.5	0.0	0.0	149.0	0.0	0.0
4795	y = 19.212x + 72.199	0.070	232.7	1.4	60.0	149.0	0.0	0.0
4766	y = 20.121x + 73.124	0.071	139.5	0.0	0.0	149.0	0.0	0.0
4769	y = 19.293x + 72.072	0.072	139.5	0.0	0.0	149.0	0.0	0.0
4802	y = 19.095x + 71.823	0.072	139.5	0.0	0.0	180.0	0.2	20.0
4776	y = 18.089x + 70.522	0.073	171.6	0.4	20.0	149.0	0.0	0.0
4777	y = 18.329x + 70.664	0.075	173.3	1.0	20.0	149.0	0.0	0.0

4808	y = 18.455x +	0.075	169.2	0.2	20.0	242.0	0.6	60.0
	70.812							
4767	y = 19.174x + 71.487	0.076	139.5	0.0	0.0	149.0	0.0	0.0
	y = 19.306x +							
4816	71.627	0.076	139.5	0.0	0.0	149.0	0.0	0.0
4922	y = 19.658x +	0.076	169.2	0.2	20.0	242.0	0.6	60.0
4823	72.009	0.076	107.2	0.2	20.0	242.0	0.6	60.0
4826	y = 19.593x +	0.076	292.1	1.8	100.0	149.0	0.0	0.0
	71.910							
4810	y = 19.425x +	0.077	139.5	0.0	0.0	149.0	0.0	0.0
	71.640 y = 20.137x +							
4784	72.312	0.078	139.5	0.0	0.0	149.0	0.0	0.0

4787	y = 19.873x + 72.038	0.078	139.5	0.0	0.0	149.0	0.0	0.0
4772	y = 20.175x + 72.196	0.079	139.5	0.0	0.0	149.0	0.0	0.0
4790	y = 18.531x + 70.466	0.079	139.5	0.0	0.0	180.0	0.2	20.0
4818	y = 19.930x +	0.080	139.5	0.0	0.0	149.0	0.0	0.0
4765	71.831 y = 19.511x +	0.084	139.5	0.0	0.0	149.0	0.0	0.0
4770	70.997 y = 19.438x +	0.084	139.5	0.0	0.0	149.0	0.0	0.0
	70.885 y = 19.874x +							
4785	71.391	0.084	139.5	0.0	0.0	149.0	0.0	0.0

4902	y = 19.944x +	0.085	139.5	0.0	0.0	149.0	0.0	0.0
4803	71.326	0.085	139.3	0.0	0.0	149.0	0.0	0.0
4001	y = 19.678x +	0.000	0(17	1.4	00.0	011.0	0.4	40.0
4801	70.684	0.089	261.7	1.4	80.0	211.0	0.4	40.0
Mean		0.057	-	0.21		-	0.04	
$d\chi^2$			142.0			120.4		

<sup>a</sup>CMM - Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" of the Universidade Federal Rural de Pernambuco (Recife, PE, Brazil). <sup>b</sup>y = percentage of mycelial growth inhibition; x = fungicide concentration. <sup>c</sup> Calculated by the concentration equation (mg L<sup>-1</sup>) for log probit analysis. <sup>d</sup> $\chi^2$  = chi-square value significant at 5% by Kruskal-Wallis test. <sup>e</sup>Media of ten repetitions (plants).