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1 # Biological Potencial of *Colletotrichum typhae* H.C Greene mycoherbicide for *Typha domingensis*

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22

23 ## Abstract

24 The anthropic interference in aquatic ecosystems, favors the disordered colonization of *T. domingensis*,
25 damaging the production of hydroelectric power and river traffic. Thus, the objective of this study was
26 to evaluate the potential of *C. typhae* as a mycoherbicide in the control of *T. domingensis*, in vitro and
27 in greenhouse. 107 samples of symptomatic *T. domingensis* leaves were collected in flooded areas of
28 rivers in Brazil, with identification and isolation of the collected fungal species. The concentration of
29 inoculum was determined to evaluate the incidence and severity of the disease, the influence of
30 temperature on mycelial growth and conidia germination, the effect of temperature and leaf wetness
31 period on *T. domingensis* infection by *C. typhae* and the host range test. The growth of the colonies of
32 *C. typhae* was higher at 25 to 30 ºC, there was no interference of the photoperiod on germination of the
33 spores, but the highest percentage of germination occurred at 17.39 ºC. The influence of environmental

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34 conditions on infection of inoculated leaves of *T. dominguensis* indicated that at 15 ºC and the period of
35 leaf wetness of 48 hours promoted the highest incidence of the disease, as well as the severity for the
36 same period of leaf wetness. The specificity test showed that *C. typhae* is specific and pathogenic to *T.*
37 *domingensis.* Being this the first report of the occurrence of this pathogen in aquatic macrophytes of
38 this species and in *T. domingensis* in Brazil.

39 **Keywords**: Biological control; Aquatic macrophytes, Leaf wetness

40

41 **Introduction**

42 *Typha domingensis* Pers. is an invasive macrophyte found in the Americas, Europe, Africa, Asia and
43 Oceania, being considered as a native species of South America, occurring throughout Brazil [1]. It is
44 propagated either by seeds or vegetatively, by rhizomes, with vigorous growth by the decomposition
45 and assimilation of organic matter as a source of nutrients, reaching about seven tons of rhizomes per
46 hectare [2]. Thus, *T. domingensis* is used as a biological filter for urban sewage, industrial effluents rich
47 in heavy metals and erosion control in drainage channels and reservoir banks [3].

48 However, anthropic interference in aquatic ecosystems favors the colonization of *T. domingensis*,
49 which may hinder the production of hydroelectric power, river traffic and agricultural irrigation [4]. In
50 the United States, this macrophyte accounts for the degradation of almost 12.000 $ha^{-1}$ of Florida
51 marshes, due its aggressive growth in response to eutrophication by nitrates and phosphates from
52 agricultural and urban waste and frequent fires [5]. In Brazil, it is estimated that the intense growth of
53 *T. domingensis* in reservoirs of the country's hydroelectric dams extends to about 300 $ha^{-1}$ in the water
54 mirror. These changes contribute to the reduction of water quality and biodiversity patterns in
55 environments colonized by this species [6].

56 Up to a certain limit, the development of aquatic vegetation can be considered harmful in several
57 ecosystems [7]. In order to reduce the environmental, social and economic impacts caused by

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58 hydrophytic plants, mechanical and biological control have been used [8,9]. The use of chemical
59 herbicides is another option to control aquatic weed macrophytes, being allowed in countries like the
60 United States, although its application is controversial in European countries and in Brazil [10]. This is
61 due to the low acceptance by society, due to the excessive use of toxic agrochemicals to different plant
62 species, besides the low number of registered products [11].

63 Thus, the use of weed control methods with a higher degree of specificity that reach only the target
64 species constitutes viable alternatives [12]. Among these, the mass production of microorganisms,
65 intended for the formulation of mycoherbicides, has been shown to be effective in weed management in
66 several parts of the world [13]. Research results have shown the efficacy of several commercial
67 mycoherbicides made with fungi of the genus *Colletotrichum*, with control rates reaching 90%, such as
68 the use of *Colletotrichum truncatum* (Schwein) in *Sesbania exaltata* (Raf.) Rydb. ex AW Hill.,
69 *Colletotrichum acutatum* (Sim.) in *Hakea sericea* Schrad. and *Colletotrichum gloeosporioides* Penz.
70 (Sacc.) in the control of *Aeschynomene virginica* L. [11].

71 Research on microorganisms in the prospection of mycoherbicides aims to establish optimal culture
72 conditions for mass and durable production of the inoculum in artificial culture [14]. The pathogen
73 should be genetically stable and specific in order to generate rapid and high disease level, with
74 consequent death or suppression of the target plant, and should not present pathogenicity to crops of
75 agricultural interest [15]. Thus, it is necessary to study the pathogen and its interaction with the host, as
76 well as the conditions that predispose the plant to the pathogen, since climate variables such as
77 temperature may influence both infection and colonization of the pathogen [16]. In addition, host
78 specificity and preference has been a criterion used in research with *Colletotrichum* species for the
79 biological control of plants. Some species of these pathogens are capable of infecting single hosts and,
80 conversely, there are also *Colletotrichum* species capable of infecting multiple host species [17].

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81 In recent years, little has been studied about these parasitic relationships between fungi and aquatic
82 weed macrophytes, however, satisfactory results were obtained in studies with the *Eichhornia crassipes*
83 Mart. Solms and *Cercospora rodmanni* [11]. Another important result was reported by Pitelli et al. [8],
84 showing that fungi of the genus *Colletotrichum* have been receiving attention as potential
85 mycoherbicides, suggesting that these pathogens have specific enzymes that promote infection and
86 degradation of the plant cell wall. Thus, the objective of this study was to evaluate the potential of *C.*
87 *typhae* as a mycoherbicide in the control of *T. domingensis*, in vitro and in greenhouse.
88

89 **Materials and Methods**

90 **Obtainment and identification of *T. dominguensis* pathogenic isolates**

91 A total of 107 leaf samples from symptomatic *T. domingensis* plants with necrotic spots were
92 collected from flooded areas of the São Francisco and Doce rivers, Brazil, from municipalities of the
93 states of Sergipe (SE), Espirito Santo (ES), Minas Gerais (MG) and Bahia (BA) (Table 1). The
94 collected material was packed in polyethylene bags and taken to the Plant Diseases Laboratory,
95 Department of Plant Pathology, Federal University of Viçosa, Viçosa, state of Minas Gerais, Brazil.
96

97 **Table 1. Description of the places of origin where symptomatically *T. domingensis* leaf samples**
98 **were collected, geographical coordinates, climate, temperature and rainfall (Source: Climate-**
99 **data. Org, 2015). Viçosa, MG, Brazil, 2018.**

| Place of origin | Latitude (S) | Longitude (W) | *Climate | Temperature (°C) | Rainfall (mm) |
|---|---|---|---|---|---|
| Pacatuba (SE) | 10º 27' 12" | 36º 39' 05" | As | 25.5 | 1,223 |
| Brejo Grande (SE) | 10º 25' 28" | 36º 27' 44" | As | 25.3 | 1,283 |
| Propriá (SE) | 10º 12' 40" | 36º 50' 25" | Aw | 25.9 | 821 |

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| | | | | | |
|---|---|---|---|---|---|
| Regência (ES) | 19º 23' 27" | 40º 04' 19" | Aw | 24.3 | 1,213 |
| Vila do Riacho (ES) | 19º 49' 12" | 40º 16' 22" | Aw | 24.5 | 1,178 |
| Barra do Riacho (ES) | 19º 49' 24" | 40º 04' 20" | Aw | 24.5 | 1,162 |
| Santa Cruz do Escalvado (MG) | 20º 14' 09" | 42º 48' 50" | Aw | 22.1 | 1,146 |
| Rio Doce (MG) | 20º 14' 41" | 42º 53' 45" | Aw | 22.2 | 1,143 |
| Ipatinga (MG) | 19º 42' 23" | 42º 34' 33" | Aw | 23.8 | 1,143 |
| São Francisco (MG) | 15º 56' 55" | 45º 51' 52" | Aw | 23.6 | 1,053 |
| Juazeiro (BA) | 09º 24' 50" | 40º 30' 10" | BSh | 24.8 | 422 |
| Paulo Afonso (BA) | 09º 24' 22" | 38º 12' 53" | BSh | 25.8 | 540 |
| Xingozinho (BA) | 09º 32' 30" | 38º 01' 59" | BSh | 25.8 | 540 |

100 *Classification according to [18]. http://pt.climate-data.org/country/114/

101 As and Aw (tropical savannah climate with dry winter season), with average temperature in any month of the
102 year exceeding 18 °C. Winter is dry, with average rainfall less than 60 mm in at least one of the months of this
103 season. BSh (hot semi-arid climate), with average temperature of the hottest month of the year 27.8 ° C. With a
104 dry season during the year, with average rainfall in the driest month around 8 mm. Most of the rainfall occurs in
105 March with an average of 83 mm.

106

107 Isolates of the collected samples were made and the fragments containing the fungal structures were
108 grown in Petri plates containing Potato Carrot Agar (PCA) culture medium. The isolates were
109 incubated at 25 ± 3 °C in the dark for eight days [19]. The identification of organisms at the genus level
110 was carried out using the identification key of Barnett and Hunter [20], by observations of the
111 structures under a stereoscopic microscope. In order to confirm the pathogenicity of the isolates, they
112 were inoculated in *T. dominguensis* disease free seedlings produced in a greenhouse, reproducing the
113 symptoms verified in the field. For this, fungal suspensions ($2.5x10^6$ conidia / mL), plus Tween 80
114 (0.05%) were used and the inoculation was done by brushing. The plants were then kept in a fog

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115 chamber at 25 ºC for 48 hours. After that time, they remained in the greenhouse for the evaluation,
116 which was carried out daily, for 30 days.

117 **Concentrations of *Colletotrichum* sp. to evaluate the incidence and severity of the disease**

118 Due to the rapid sporulation in culture medium and the severity of the disease, which was assessed
119 by fast evolution of the symptoms in the inoculated plant, only the cultures of the genus *Colletotrichum*
120 sp. obtained in the detection test were used. To this end, the isolates were grown in potato dextrose agar
121 (PDA) culture medium and incubated at 25 ± 1 °C in the dark for eight days. The identification of the
122 *Colletotrichum typhae* species was carried out under a stereoscopic microscope [21], being this the first
123 report of the occurrence of this pathogen in aquatic macrophytes of this species and in *T. domingensis*
124 in Brazil. The sample was subcultured three times to obtain a pure culture and stored at 5 ± 1 °C after
125 characterization of the species for further testing.

126 Four groups of adult and young plants of *T. domingensis*, arranged in three replicates, were used to
127 evaluate the severity of the disease, with each replication consisting of a pot with three plants. The
128 leaves were inoculated by brushing the conidial suspensions with 0.05% Tween 80 fixative, adjusted at
129 four different concentrations (one for each group of plants): 2.5 x $10^4$, 2.5 x $10^5$, 2.5 x $10^6$ and 2.5 x $10^7$
130 conidia / mL $^{-1}$. The experiment was conducted in a humid chamber at 25 ± 1 °C at 90% relative
131 humidity and transferred to a greenhouse after 48 hours. The evaluations were carried out daily, during
132 eight days. Since there is no descriptive or diagrammatic scale of the disease, the severity was
133 evaluated by the percentage of leaf area affected by the symptoms, which was quantified from
134 photocopied detached symptom leaves [22] using the software Severity.exe., where the injured area
135 was calculated, thus it was possible to correlate the symptomatology and leaf morphology with
136 damages caused by the anthracnose disease caused by *C. typhae*.
137

138 **Influence of temperature on mycelial growth and germination of *C. typhae* conidia**

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139 For evaluation of mycelial growth, *C. typhae* mycelium discs (RWB99) from colonies with eight days
140 of incubation in Vegetable Broth Agar (VBA) medium were placed in Petri plates, containing the same
141 medium, one disc per plate, with five replicates with the experimental unit consisting of two Petri
142 plates incubated at temperatures of 15, 20, 25, 30 and 35 °C in the dark. The evaluations were
143 performed daily by measuring the diameter of the colonies in two perpendicular directions, during four
144 days and from the averages obtained it was calculated the area below the mycelial growth curve.
145 To evaluate the conidia germination, a $2.5x10^6$ conidia / mL suspension of *C. typhae* was used. Four
146 aliquots of 50µL were deposited at equidistant points in Petri plates containing agar-water medium
147 scattered with a Drigalski loop. Five replicates were used, each containing two plates. The plates were
148 incubated for eight days under the same conditions of the tests for evaluation of mycelial growth. After
149 this time, aliquots of sterile distilled water were added to each plate. A fungal suspension was prepared
150 by scraping the colonies and 100 spores of each plate were counted with the aid of a hemacytometer
151 and an optical microscope, quantifying, in percentage, how many of these spores were germinated.
152

153 **Influence of temperature and leaf wetness period on *T. domingensis* infection by *C. typhae***

154 *T. domingensis* seedlings were prepared in polyethylene pots with a three liter capacity using coarse
155 sand (3.0 µm), organic substrate and Red Latosol (1:1:1; v/v) as substrate. Four groups of fifteen plants
156 of different ages were inoculated with a suspension of $2.5 \times 10^6$ spores / ml by atomization until run-off
157 with P-600 compressor with the "PULVERJET" P-110 pistol. After inoculation the plants were kept in
158 a humid chamber (wire-frame vessels wrapped with internally moistened plastic) within growth
159 chambers with the following temperatures: 15, 20, 25 and 30 ± 2 °C. Each group consisted of five
160 subgroups of three plants, which were gradually removed from the wraps after 8, 12, 24 and 48 hours
161 of leaf wetness. A subgroup for each treatment was placed in each growth chamber without being
162 wrapped in plastic (corresponding to zero hour of leaf wetness). After completing 48 hours, all plants

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163 were taken to the greenhouse to evaluate the incidence and severity of the disease. Six evaluations were
164 performed at two-day intervals, with the first evaluation on the eighth day after inoculation.
165

166 ***C. typhae* host range**

167 In order to verify the host range of the isolate under study, inoculations were carried out on the species
168 selected according to the phylogenetic centrifugal method [23], with modifications. Due to difficulties
169 of obtaining other plants of the same genus and restrictions of the distribution of the Typhaceae family
170 or even of the Typhales order, made it difficult to perform more complete analyzes for this pathogen.
171 Thus, *C. typhae* inoculation was performed in *T. domingensis* and plants of the same order and related
172 orders of fruit, forest and large crop species (Anacardiaceae, Apiaceae, Araceae, Arecaceae,
173 Asteraceae, Bromeliaceae, Cannaceae, Caricaceae, Chenopodiaceae, Commelinaceae, Cyperaceae,
174 Euphorbiaceae, Euriocaulaceae, Fabaceae, Halaceae, Laceaceae, Malaceae, Mayaceae, Maraceaeae,
175 Musaceae, Myraceceae, Poaceae, Pontederiaceae, Rosaceae, Rubiaceae, Rutaceae, Solanaceae,
176 Sparganiaceae, Strelitzaceae, Typhaceae, Vitaceae, Xyridaceae and Zingiberaceae). Forest species and
177 agricultural crops were included in the evaluation, whether or not related to the target plant, and plants
178 of species susceptible to fungi of the genus *Colletotrichum* sp., totaling 53 species. Ten leaves of each
179 plant species were inoculated by the $2.5 \times 10^6$ conidia / mL $^{-1}$ suspension by the brushstroke method
180 and were kept in a humid chamber at 25 ± 1 °C with 90% relative humidity for 48 hours. After this
181 time, they were transferred to a greenhouse and the evaluations to detect the presence or absence of
182 symptoms caused by *C. typhae* were carried out at the 3rd, 7th and 21st days.
183

184 **Statistical analysis**

185 The tests performed to identify the effect of temperature on mycelial growth and germination of *C.*
186 *typhae* spores were conducted in a randomized complete design with five replicates, with two Petri

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187 plates each. To evaluate the influence of leaf wetness and temperature on *T. dominguensis* infection by
188 *C. typhae*, the trials were conducted in a randomized complete design with 20 treatments and three
189 replicates, each replicate consisting of a pot with three plants. The statistical editor used for the
190 analyzes was SAEG**.**

191 The effect of the temperatures was evaluated by the analysis of variance and linear regression
192 analysis. The equations were chosen based on the significance of the regression coefficients, adopting
193 the level of 1% of probability. In the photoperiod effect test, the treatment means were compared by the
194 Tukey test at a significance level of 5%.

195 In order to evaluate the effect of temperature and leaf wetness on the incidence and severity of the
196 disease, a experiment was conducted in a complete randomized design, with four temperatures and five
197 leaf wetness periods, treated in a factorial scheme with two factors (temperature and leaf wetness
198 periods) and the data submitted to a linear regression analysis.

199

200 **Results and Discussion**

201 **Effect of different temperatures on *C. typhae* conidia germination**

202 The microflora associated with *T. dominguensis* plants was identified as *Colletotrichum typhae*
203 (RWB-99, CBM-24, 26, 35, 94 and 97), *Cercospora* sp. (CBM-32, 97), *P. dichota* (CBM-14, 16, 24,
204 26, 94, 97), *Phoma* sp. (CBM-05, 97), *C. typharum* (CBM-5, 13, 14, 16, 23, 32, 97, 24) and *Stenella*
205 sp. (CBM-13), but only the *C. typhae* isolates were pathogenic to *T. domingensis* at the concentration
206 used in the pathogenicity test. For this reason, the other tests were carried out only with *C. typhae*, to
207 verify their bioherbicidal capacity on *T. dominguensis*.

208 After analyzing the pathogenicity of the isolate at different inoculum concentrations, a development
209 pattern of the anthracnose symptoms was observed at the concentration of 2.5 x $10^6$ spores / ml on the
210 eighth day after inoculation, thus, the infection started with lesions slightly visible although

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211 homogeneously distributed in the lap of the plants reaching a leaf area of 19%. In this study, levels
212 from 0 to 100% of injured leaf area were identified, and the images obtained aided in the evaluation of
213 the other trials.

214 The conidia produced by *Colletotrichum* sp. are survival structures. They are important in infection
215 of the host and in the propagation of these pathogens. Despite its relevance to the fungal life cycle, the
216 conidia biology has not been extensively investigated. In this study, the first description of the *C.*
217 *typhae* conidia germination, whose ideal thermal condition was verified at a temperature of 20 °C, was
218 observed, whereas the lowest spore production index was observed at the temperature of 35 °C (Fig.
219 1A).

220 **Figure 1 – Effect of different temperatures, on *C. typhae* conidia germination in agar-water**
221 **(A) and micelial growth in VBA (B).**

222 Similar results were obtained by Estrada et al. [24] who found that the optimal temperature for
223 sporulation of *C. gloeosporioides* was between 20 and 25 °C. However, Poltronieri et al. [25] reported
224 that the optimal temperature for conidia germination may vary for different species of the genus
225 *Colletotrichum* sp., Harding and Raizada [11] observed that the germination of *Colletotrichum musae*
226 B & MAC conidia is stimulated between 27 and 30 °C while Couto and Menezes [26], demonstrated
227 that the *Colletotrichum coccodes* Wallr. sporulation occurs between 20 and 30 °C and for
228 *Colletotrichum lagenarium* (Pass.) Ellis & Halst. at 16 °C.

229 The temperature data on the disease incidence were adjusted to the cubic regression model for
230 mycelial growth and conidial germination variables. The conidia germination and the temperature
231 range between 25 and 30 ºC provided the highest *C. typhae* mycelial growth (Fig. 1A and 1B).

232 The inhibitory effect of temperature on fungi growth is variable, but most pathogens show better
233 development at 20 to 25 °C. For *C. acutatum*, McKay et al. [27] reported that the optimum temperature
234 for mycelial growth was 25 °C, and Harding and Raizada [11] stated that elevated temperatures around

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235 35.5 °C paralyze *C. gloeosporioides* mycelial growth, corroborating with the results obtained in this
236 study.
237 The relationship of a pathogen to the host may vary from one host to another and in this case, the
238 interactions between this pathogenetic system are still not well understood [17]. Due to this interaction,
239 studies on microorganisms for biological control of plants, aim to establish ideal culture conditions for
240 mass and durable production of the inoculum in artificial culture [14]. Climate variables such as
241 temperature, for example, influences the rate of fungal reproduction, physiological conditions of the
242 host, growth and aggressiveness of pathogens. Thus, the knowledge of the interaction of the pathogen
243 with environmental factors has a practical meaning, since the environment can alter its pathogenicity
244 [28].
245 **Effect of leaf wetness on the incidence of the desease and severity caused by *C. typhae***
246 The disease incidence data, when submitted to different temperatures and leaf wetness periods, were
247 adjusted to the linear regression model, being a decreasing incidence in relation to the increase in
248 temperature, and increasing in relation to the leaf wetness period (Fig 2 A and B). The temperature
249 influenced the formation of chlorotic lesions on the leaf blade surface of *T. domingensis* plants, with
250 the exception of 20% of the plants kept at temperatures of 15, 20 and 30 °C, which had no symptoms of
251 anthracnose in the first evaluation that occurred eight days after inoculation. It was also found that at
252 temperatures of 15 and 20 °C, the maximum incidence of the disease was 95 and 90%, respectively
253 (Fig 2A). On the other hand, during the evaluations of leaf wetness periods, it was observed that after
254 the period of 48 hours, in addition to the influence of the temperature, the duration of the leaf wetness
255 period favored the incidence of *C. typhae* with maximum percentage rate of 95% of symptomatic plants
256 (Fig 2B).
257

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258 **Figure 2 – Effect of leaf wetness on the incidence of the desease caused by *C. typhae* em plants of**
259 ***T. domingensis,* in the greenhouse.**

260

261 With regards to the severity of the disease, it was observed that at zero hour of leaf wetness, small
262 lesions near the lap of the plants were contacted, whereas in the higher parts of the plants these lesions
263 were very scarce (Fig 3). Lima et al. [29] evaluating the development of phytopathogens, reported the
264 absence of disease in zero wetness, indicating that the incidence of the fungus *Puccinia kuehnii* may be
265 related to the need of free water on the leaves of the inoculated plants and a wetness period for at least
266 12 hours for fungal infection to occur. However, by the results obtained in this study, probably the
267 presence of anthracnose symptoms in the lap of *T. domingensis* plants at zero wetness period with a
268 rate of 85% is due to the proximity of these areas to the water in the pot, where it was possible to create
269 a microclimate that has favored conidial germination, infection and lesion development.

270

271 **Figure 3 – Effect of leaf wetness on the severity of the desease caused by *C. typhae* in plants of *T.***
272 ***domingensis*, in the greenhouse.**

273

274 Spores of most pathogenic fungi require free water for hours at the host surface for germination and
275 consequent penetration. The increase in disease intensity accompanies the wetness period up to a
276 certain threshold, from which the maximum amount of disease is reached and it is no longer influenced
277 by additional wetness periods [30]. According to Lima et al. [29], from a certain concentration of
278 infection points, lesions that form first, may hinder the development of others that result from
279 subsequent infections. However, in the present study the highest progression of both disease incidence
280 and severity was detected with increasing leaf wetness time duration (Figs 2B and 3).

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281 The highest severity of symptoms detected was observed in 8, 24 and 48 hours leaf wetness periods,
282 with rates of 82, 85 and 88%, respectively. There is little research on the epidemiology of anthracnose
283 caused by *C. typhae*; in addition, the results obtained in this study are the first report of this disease in
284 *T. domingensis* plants, so further studies are probably needed to confirm these results (Fig 3).
285 Climate variables such as temperature and precipitation influence the development of anthracnose
286 symptoms. Under high temperature conditions, pathogens can grow rapidly and severe outbreaks of
287 disease are expected in environments with high relative humidity [31]. According to Soares-Colletti
288 and Lourenço [32], the infection rate is high in the range of 15 to 30 ºC for *C. gloeosporioides*, with the
289 ideal temperature around 25 ºC, however, for *C. acutatum* as for *C. typhae* a maximum incidence of
290 this pathogen occurred at 20 °C. Regarding the wetness period, the results obtained in this study with
291 *C. typhae* coincide with those found by Soares, Lourenço and Amorim [33], who reported that the
292 greater severity of anthracnose caused by *C. acutatum* and *C. gloeosporioides* was high after 48 hours.
293 From the results obtained in this study during the evaluation of the incidence and severity of
294 anthracnose in plants of *T. domingensis*, it was verified that the amount of hours of the wetness period
295 needs to be continuous reaching its peak after 48 hours. In order to cause infection in plants, *C. typhae*
296 probably requires several short wetness rather than a single long period. Among the factors that have
297 most restricted the good performance of potential mycoherbicides is the need for a long duration of the
298 leaf wetness period for the infection to occur [34]. However, these restrictions can be overcome by
299 methods of artificial inoculation, since the moisture level and inoculation during the leaf wetness
300 period can be increased by the more efficient use of wetness agents or emulsions, or by applying the
301 fungus to gel or by using granular preparations [35,36].
302 Most of the studied potential plant mycoherbicides have been based on formulations of fungi
303 species. The biological control that is involved in the application of fungal spores propagation in
304 concentrations that do not occur in nature, is the most used strategy in the use of mycoherbicides based

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305 on these pathogens [37]. Several studies indicate that fungi of the genus *Colletotrichum* receive
306 attention as potential mycoherbicides, since they have enzymes that degrade the plant cell walls,
307 suggesting that some of these proteins may have specific roles in plant infection. There is also evidence
308 that both species of *Colletotrichum* have the ability to produce indole-acetic acid, whose derivatives are
309 well-established models of herbicides [38].

310 However, anthracnose caused by species of the genus *Colletotrichum* is a common and destructive
311 disease in diverse agricultural crops and forest species. Its global occurrence causes significant losses
312 in tropical and subtropical regions [39]. Although the genus contains species with different lifestyles,
313 plants can be infected during any stage of development and symptoms appear after colonization of
314 fungi, which is characterized by necrotic lesions in leaves [40]. Therefore, due to the lack of
315 information about its occurrence and aggressiveness, there is a need to determine the susceptibility of
316 plant species to validate the prospection of mycoherbicides, adopting the pathogenicity test proposed
317 by Wapshere [23], widely used in biological control programs.

318 **Pathogenicity of the Colletotrichum hyphae isolate inoculated at room temperature**

319 In this study, the host range was evaluated using 53 plants, among species and / or varieties included
320 in the test, and only *T. domingensis* was susceptible to *C. typhae*, suggesting high specificity for the
321 analyzed isolate (Table 2). The *C. typhae* isolate obtained in the survey produced symptoms similar to
322 those obtained in the collection areas, with lesions on the leaves, both in the adaxial and abaxial parts,
323 punctiform, yellow with chlorotic halo, becoming necrotic to the center with a light brown color. Based
324 on these results, it can be inferred that this pathogen has effects mainly on the aerial part of *T.*
325 *domingensis*, therefore, it is necessary to carry out more studies mainly at the molecular level, in order
326 to confirm its mycoherbicide potential. It should be considered that a mycoherbicide may not
327 necessarily have the same effect on plants as a chemical herbicide. However, these compounds have
328 the potential to provide a competitive advantage for seedling growth through infection and growth

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329 retardation of weed seedlings. Therefore, isolation and structural characterization and mode of action of
330 phytotoxins produced by pathogenic fungi for weeds, including aquatic invasive plants, should be
331 investigated [41].

332 **Table 2. Pathogenicity of the ecogenicidade Colletotrichum hyphae isolate inoculated at room**
333 **temperature in humid chamber.Viçosa, MG, Brazil, 2018.**

| Family | Species/Variety | Result[*] |
|---|---|---|
| Anacardiaceae | *Mangifera indica* L./ Ubá | - |
| Apiaceae | *Daucus carota* L./Brasília | - |
| Araceae | *Pistia stratiotes* L. | - |
| Arecaceae | *Cocus nucifera* L./ Anã | - |
| Asteraceae | *Helianthus annuus* L. /UFV-10 | - |
| Bromeliaceae | *Ananas comosus* (L.) Merr./ Pérola | - |
| Cannaceae | *Cana denudata* Rosc. | - |
| Caricaceae | *Carica papaya* L. /Formosa | - |
| Chenopodiaceae | *Beta vulgaris* L./Wonder | - |
| Commelinaceae | *Commelina bengalensis* L. | - |
| | *Tradescantia zebrina* Hort. ex Bosse. | - |
| | *Tradescantia pallida* (Rose) Hunt. | - |
| | *Callisia repens* (Jacq) L. | - |
| | *Commelina erecta* L. | - |
| Cyperaceae | *Cyperus esculentus* L. | - |
| | *Cyperus rotundus* L. | - |

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| | | |
|---|---|---|
| Euphorbiaceae | *Manihot esculenta* Crantz. | - |
| | *Hevea brasiliensis* Willd. | - |
| Euriocaulaceae | *Paepalanthus* sp. | - |
| Halorragaceae | *Myriophyllum aquaticum* (Vell.) Verdc | - |
| Fabaceae | *Glycine max* L. / UFV-16 | - |
| | *Phaseolus vulgares* L. | - |
| | *Rachis hypogaea L. /* IAC-Jimbo | - |
| Juncaceae | *Eleocharis filiculmis* Kunth. | - |
| Lauraceae | *Persea americana* Mill. / Wagner | - |
| Liliaceae | *Allium cepa* L. / Monte Alegre | - |
| Malvaceae | *Gossypium hirsutum* L. / IAPAR 4PR.1 | - |
| | *Gossypium hirsutum* L. / IAC - 22 | - |
| Mayacaceae | *Mayaca* sp. | - |
| Marantaceae | *Marantha* sp. | - |
| Moraceae | *Ficus carica* L. / Roxo de Valinhos | - |
| Musaceae | *Musa* sp./Prata | - |
| Myrtaceae | *Psidium guajava* L. / Campos | - |
| Poaceae | *Oryza sativa* L. / BR IRGA 409 | - |
| | *Oryza sativa* L. / CICA-8 | - |
| | *Echinochloa polystachya* (H.B.K.) Hitch | - |
| | *Saccharum* sp. / Cana Caiana | - |

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| | | |
|---|---|---|
| | *Zea mays* L. | - |
| | *Sorghum bicolor* (L.) Moench/ BR - 700 | - |
| Pontederiaceae | *Eichhornia azurea* (Swartz) Kunth | - |
| | *Eichhornia crassipes* (Mart.) Solms | - |
| Rosaceae | *Prunos* sp / REDHAVEN | - |
| Rubiaceae | *Coffea arabica* L. / CATUAI - 2144 | - |
| Rutaceae | *Citrus* sp. / Taiti | - |
| Solanaceae | *Nicotiana tabacum* L. / XALTHI | - |
| | *Lycopersicon sculentum* Mill. /Santa Clara | - |
| | *Capsicum annuum* L. / Casca Dura | - |
| Sparganiaceae | *Sparganicum sp.* | - |
| Strelitzaceae | *Strelitzia reginae* Banks | - |
| Typhaceae | *Typha domingensis* Pers. | + |
| Vitaceae | *Vitis* sp. /Niagara Rosada | - |
| Xyridaceae | *Xyris sp* | - |
| Zingiberaceae | *Zingiber officinale* Rosc. | - |

334 * (+) presence of symptoms and signs of the pathogen, (-) absence of symptomatology.

335

336 Rangel-Peraza et al. [2] considered submerged aquatic plants as the most problematic, since they
337 drastically reduce water flow, are rapid in invasion of new areas and difficult to manage or control. The
338 perennial growth habit and the formation of monophytic colonies seem to make them the ideal target of

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339 chemical control. However, the use of chemical herbicides is often compromised by problems related
340 to the aquatic environment, including dilution and contact time in tap water.
341

## 342 Conclusions

343 The growth of the *C. typhae* colonies in artificial medium is higher when they are submitted to
344 temperatures of 25 to 30 ºC, whereas, for spore germination, there was no photoperiod interference, but
345 the highest percentage of germination occurred at 17.39 ºC. The influence of environmental conditions
346 on the infection in artificially inoculated leaves of *T. domingensis* indicated that the temperature of 15
347 ºC and 48 hours of leaf wetness promoted the highest disease incidence, as well as the severity for the
348 same period of leaf wetness. The specificity test showed that *C. typhae* is very specific since it was
349 pathogenic only to *T. domingensis*. Being this the first report of the occurrence of this pathogen in
350 aquatic macrophytes of this species and in *T. domingensis* in Brazil.

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373 **References**

374 **1.** Oliveira JPV, Pereira MP, Duarte VP, Corrêa FF, Castro EM, Pereira FJ. Cadmium tolerance of
375 *Typha domingensis* Pers. (*Typhaceae*) as related to growth and leaf morphophysiology. Braz J
376 Biol. 2018;77(1):52-9. https://dx.doi.org/10.1590/1519-6984.171961.

377 **2.** Rangel-Peraza JG, Mendivil-García K, Cedillo-Herrera CIG, Rochín-Medina JJ, Rodríguez-
378 Mata A E, Bustos-Terrones YA. Optimization of organic matter degradation kinetics and
379 nutrient removal on artificial wetlands using *Eichhornia crassipes* and *Typha domingensis*.
380 Enviro Technol. 2017;15(2):1-9. https://doi.org/10.1080/09593330.2017.1400111

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381 **3.** Bonanno G, Cirelli GL. Comparative analysis of element concentrations and translocation in
382 three wetland congener plants: *Typha domingensis*, *Typha latifolia* and *Typha angustifolia.*
383 Ecoto and Enviro Saf. 2017;143(5): 92-101. https://doi.org/10.1016/j.ecoenv.2017.05.021
384 **4.** Lagerwall G, Kiker G, Muñoz-Carpena R, Wang N. Accounting for the impact of
385 management scenarios on *Typha Domingensis* (Cattail) in an everglades wetland. Enviro
386 Manage. 2017;59(1):129-40. https://doi.org/10.1007/s00267-016-0769-0
387 **5.** Smith SM, Newman S. Growth of southern cattail (*Typha domingensis* Pers.) seedlings in
388 response to fire-related soil transformations in the Northern Florida everglades. Wetlands.
389 2001;21(3):363-9.
390 **6.** Pompêo M. Monitoramento e manejo de macrófitas aquáticas em reservatórios tropicais
391 brasileiros. Instituto de Bio- ciências da USP. 2017;138.
392 https://doi.org/10.11606/9788585658670. Portuguese.
393 **7.** Malaspina IC, Cruz C, Garlich N, Bianco S, Pitelli RA. Effectiveness of diquat, both isolated
394 and associated with copper sources in controlling the *Hydrilla verticillata* submerged
395 macrophytes and ankistrodesmus gracilis microphyte. Planta Daninha. 2017;35(3):1-8.
396 https://dx.doi.org/10.1590/s0100-83582017350100018.
397 **8.** Pitelli RLCM, Toffanelli C, Vieira E, Pitelli RA, Velini ED. Dinâmica da comunidade de
398 macrófitas aquáticas no reservatório de Santana, RJ. Planta Daninha. 2008;26(3):473-480.
399 Portuguese.
400 **9.** Diao YZ, Fan JR, Wang ZW, Liu XL. First Report of *Colletotrichum boninense* causing
401 anthracnose on pepper in China. Plant Dis. 2013;97(1):138-148. https://doi.org/10.1094/PDIS-
402 04-12-0403-PDN

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403 **10.** Garlich N, Cruz C, Silva A, Carraschi SP, Malaspina IC, Pitelli RA, et al. Diquat associated
404 with copper sources for algae control: efficacy and ecotoxicology. J Environ Sci Health B.
405 2016;1(4):1-7. https://doi.org/10.1080/03601234.2015.1120611
406 **11.** Harding DP, Raizada MN. Controlling weeds with fungi, bacteria and viruses: a review. Front
407 Plant Sci. 2015;6(3):1-14. https://doi.org/10.3389/fpls.2015.00659
408 **12.** Heydari A, Pessarakli M. A review on biological control of fungal plant pathogens using
409 microbial antagonists. J Biol Sci. 2010;10(4):273-90.
410 https://doi.org/10.3923/jbs.2010.273.290
411 **13.** Singh B, Saxena S, Meshram V, Kumar M. Mycoherbicidal potential of *Phaeoacremonium*
412 *italicum*, a new pathogen of *Eichhornia crassipes* infesting harike wetland, India. Mycobiol.
413 2016;44(2):85-2. https://doi.org/10.5941/MYCO.2016.44.2.85
414 **14.** Jiménez MM, Villasana AM. Evaluation of toxicity of *Cercospora piaropi* in a mycoherbicide
415 formulation by using bacterial bioluminescence and the Ames mutagenicity tests.
416 Mycopathologia. 2009;167(4):203-8. https://doi.org/10.1007/s11046-008-9161-9
417 **15.** Cimmino A, Masi M, Evidente M, Superchi S, Evidente A. Fungal phytotoxins with potential
418 herbicidal activity: chemical and biological characterization. Nat Prod Reports.
419 2015;32(12):1629-53. https://doi.org/10.1039/C5NP00081E
420 **16.** Kusari S, Hertweck C, Spiteller M. Chemical ecology of endophytic fungi: origins of secondary
421 metabolites. Chem Biol. 2012;19(7):792-8. https://doi.org/10.1016/j.chembiol.2012.06.004
422 **17.** Doyle VP, Oudemans PV, Rehner SA, Litt A. Habitat and host indicate lineage identity in
423 *Colletotrichum gloeosporioides* s.l. from Wild and agricultural landscapes in North America.
424 PLoS One. 2013;8(5): e62394. https://doi.org/10.1371/journal.pone.0062394
425 **18.** Köppen W, Geinger R. Klimate der erde. Gotha: verlag justus perthes. Wall-map.1928.

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426 **19.** Villa-Rivera MG, Conejo-Saucedo U, Lara-Marquez A, Cano-Camacho H, Lopez-Romero E,
427 Zavala-Paramo MG. The Role of Virulence Factors in the Pathogenicity of *Colletotrichum* sp.
428 Curr Protein Pept Sci. 2017;18(10):1005-18.
429 https://doi.org/10.2174/1389203717666160813160727
430 **20.** Barnett HC, Hunter BB. Illustrated genera of imperfect fungi. 3th ed. Burgess:
431 Mineapolis;1972.
432 **21.** Greene, HC. Notes on Wisconsin parasitic fungi. XXII. Transactions of the Wisconsin
433 Academy of Science and Arts, Letters. 1956;44(41): 29-43.
434 **22.** Horsfall JG, Barratt RW. An improved grading system for measuring plant diseases.
435 Phytopathol. 1945;35(5): 655.
436 **23.** Wapshere AJA. Strategy for evaluating the safety of organisms for biological weed control.
437 Ann Apl Biol. 1974;77(5):201-11. https://doi.org/10.1111/j.1744-7348.1974.tb06886.x
438 **24.** Estrada AB, Dodd JC, Jeffries P. Effect of humidity and temperature on conidial germination
439 and appressorium development of two Philippine isolates ofthe mango anthracnose pathogen
440 *Colletotrichum gloeosporioides*. Plant Pathol. 2000;49(5):608-18.
441 https://doi.org/10.1046/j.1365-3059.2000.00492.x
442 **25.** Poltronieri TPS, Azevedo LAS, Silva DEM. Efeito da temperatura no crescimento micelial,
443 produção e germinação de conídios de *Colletotrichum gloeosporioides*, isolados de frutos de
444 palmeira Juçara (*Euterpe edulis* Mart). Summa Phytopathol. 2013;39(4):281-285. Portuguese.
445 **26.** Couto EF, Menezes M. Physiological and morphological characterization of *Colletotrichum*
446 *musae* isolates. Fitopatol Bras. 2004;29(4):406-12. https://dx.doi.org/10.1590/S0100-
447 41582004000400008
448 **27.** Mckay SF, Freeman S, Minz D, Maymon M, Sedgley M, Collins GC, Scott ES. Morphological,
449 genetic, and pathogenic characterization of *Colletotrichum acutatum*, the cause of anthracnose

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450 of almond in Australia. Phytopathol. 2009;99(8):985-95. https://doi.org/10.1094/PHYTO-99-8-
451 0985

452 **28.** Terefe H, Fininsa C, Sahile S, Tesfaye K. Effect of temperature on growth and sporulation of
453 *Botrytis fabae*, and resistance reactions of faba bean against the pathogen. J Plant Pathol
454 Microbiol. 2015;6(7): 1-9. https://doi.org/10.4172/2157- 7471.1000285

455 **29.** Lima LL, Scaloppi EAG, Barreto LF, Barreto M. Temperatures and leaf wetness duration on
456 orange rust development in sugarcane (*Puccinia kuehnii*). Summa Phytopathol. 2017;43(2):132-
457 5. https://dx.doi.org/10.1590/0100-5405/1890

458 **30.** Bedendo IP, Amorim L. Ambiente e doença. In: Amorim L, Rezende JAM, Bergamin Filho, A.
459 Manual de Fitopatologia: Princípios e conceitos. Ceres: São Paulo; 2011. p. 133-47.

460 **31.** Moral J, Jurado-Bello J, Sánchez, M I, Oliveira R, Trapero A. Effect of temperature, wetness
461 duration, and planting density on olive anthracnose caused by *Colletotrichum* spp. Phytopathol.
462 2012;102(10):974-81. https://doi.org/10.1094/PHYTO-12-11-0343

463 **32.** Soares-Colletti AR, Lourenço SA. Effect of temperature, wetness duration and cultivar on the
464 development of anthracnose in guava fruits. Summa phytopathol. 2014;40(4):307-312.
465 http://dx.doi.org/10.1590/0100-5405/1988

466 **33.** Soares AR, Lourenço SA, Amorim L. Infecção de goiabas por *Colletotrichum gloeosporioides*
467 e *Colletotrichum acutatum* sob diferentes temperaturas e períodos de molhamento. Trop Plant
468 Pathol. 2008;33(4):265-72. http://dx.doi.org/10.1590/S1982-56762008000400002. Portuguese.

469 **34.** Boyetchko SM, Rosskopf EN, Caesar AJ, Charudattand R. Biological weed control with
470 pathogens: search for candidates to applications. Appl l Mycol and Biotechnol. 2002;2(3): 239-
471 74. https://doi.org/10.1016/S1874-5334(02)80013-2

472 **35.** Walker HL, Connick WJ. Sodium alginate for production and formulation of mycoherbicides.
473 Weed Sci. 1983;31(4):333-8. https://doi.org/10.1017/S0043174500069113

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474 **36.** Walker HL. Granular formulation of *Alternaria macrospora* for control of spurred anoda
475 (*Anoda cristata*). Weed Sci. 1981;29(3):342-5.
476 **37.** Brun T, Rabuske JE, Todero I, Almeida TC, Dias Junior JJ, Ariotti G, et al. Production of
477 bioherbicide by *Phoma* sp. in a stirred-tank bioreactor. 3 Biotech. 2016;6(2):1-9.
478 https://doi.org/10.1007/s13205-016-0557-9
479 **38.** Wei W, Ying X, Wenjun Z, Nancong W, Guogen Y, Fang P. *Colletotrichum higginsianum*
480 mitogen-activated protein kinase chmk1: role in growth, cell wall integrity, colony
481 melanization, and pathogenicity. Front Microbiol. 2016;7(3):1212-20.
482 https://doi.org/10.3389/fmicb.2016.01212
483 **39.** Araújo NAF, Vieira JDM, Moura MR, Pessoa WRLS, Barguil BM. Pathogenicity and
484 aggressiveness of *Colletotrichum gloeosporioides* isolates in ornamental pepper. Pesq. Agropec.
485 Tropical. 2016;46(3): 321-326. http://dx.doi.org/10.1590/1983-40632016v4641483
486 **40.** Villa-Rivera MG, Conejo-Saucedo U, Lara-Marquez A, Cano-Camacho H, Lopez-Romero E,
487 Zavala-Paramo MG. The Role of Virulence Factors in the Pathogenicity of *Colletotrichum* sp.
488 Curr Protein Pept Sci. 2017;18(10):1005-18.
489 https://doi.org/10.2174/1389203717666160813160727
490 **41.** Souza ARC, Baldoni DB, Lima J, Porto V, Marcuz C, Machado C, Ferraz RC, et al. Selection,
491 isolation, and identification of fungi for bioherbicide production. Braz J Microbiol.
492 2017;48(1):101-8. https://dx.doi.org/10.1016/j.bjm.2016.09.004

**Fig 1. Effect of different temperatures, on *C. typhae* conidia germination in (A) agar-water and (B) micelial growth in VBA.**

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Fig 1.

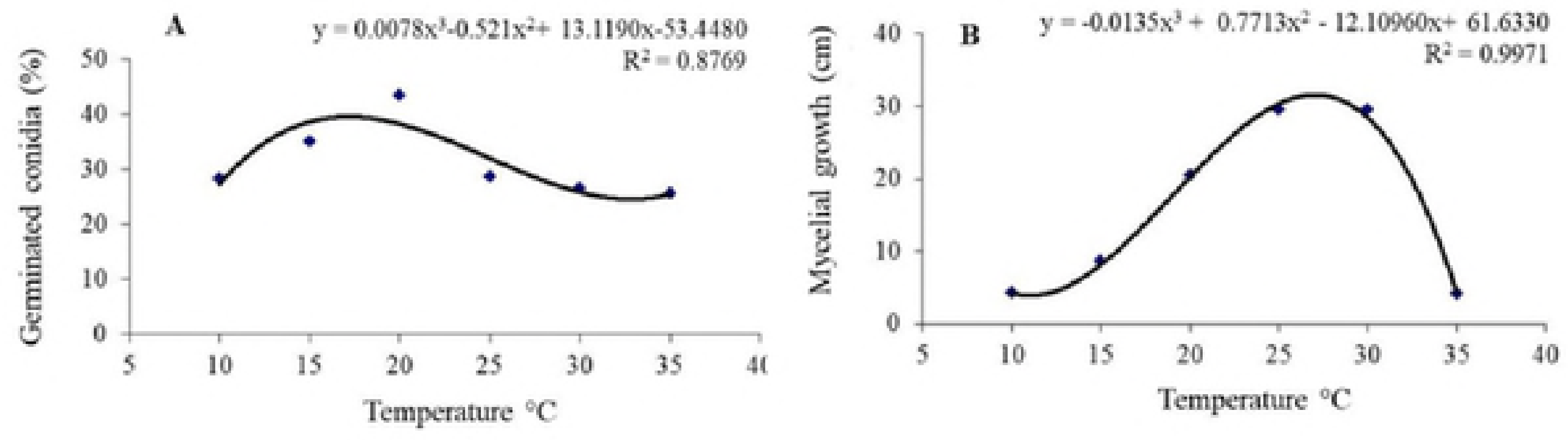


Figure 1 – Effect of different temperatures, on *C. typhae* conidia germination in agar-water (A) and micelial growth in VBA (B).

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Fig 1

**Fig 2. Effect of leaf wetness on the incidence of the desease caused by *C. typhae* em plants of *T. domingensis*, in the greenhouse.**

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Fig 2.

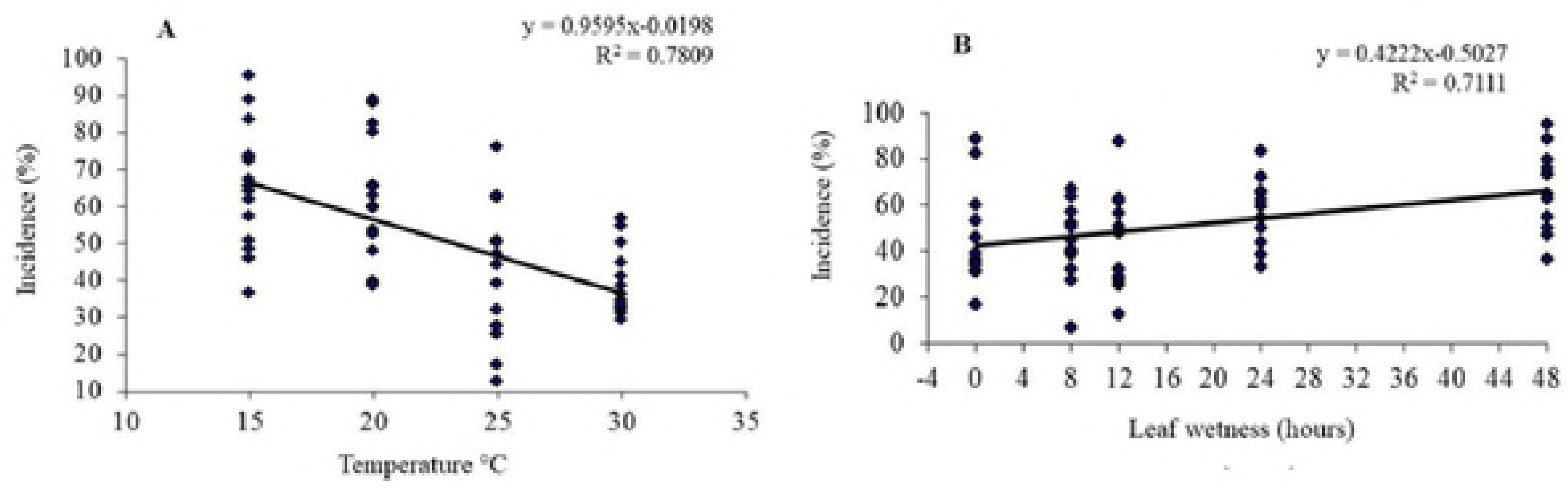


Figure 2 – Effect of leaf wetness on the incidence of the desease caused by *C. typhae* em plants of *T. domingensis*, in the greenhouse.

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Fig 2

**Fig 3. Effect of leaf wetness on the severity of the desease caused by *C. typhae* in plants of *T. domingensis*, in the greenhouse.**

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Fig 3.

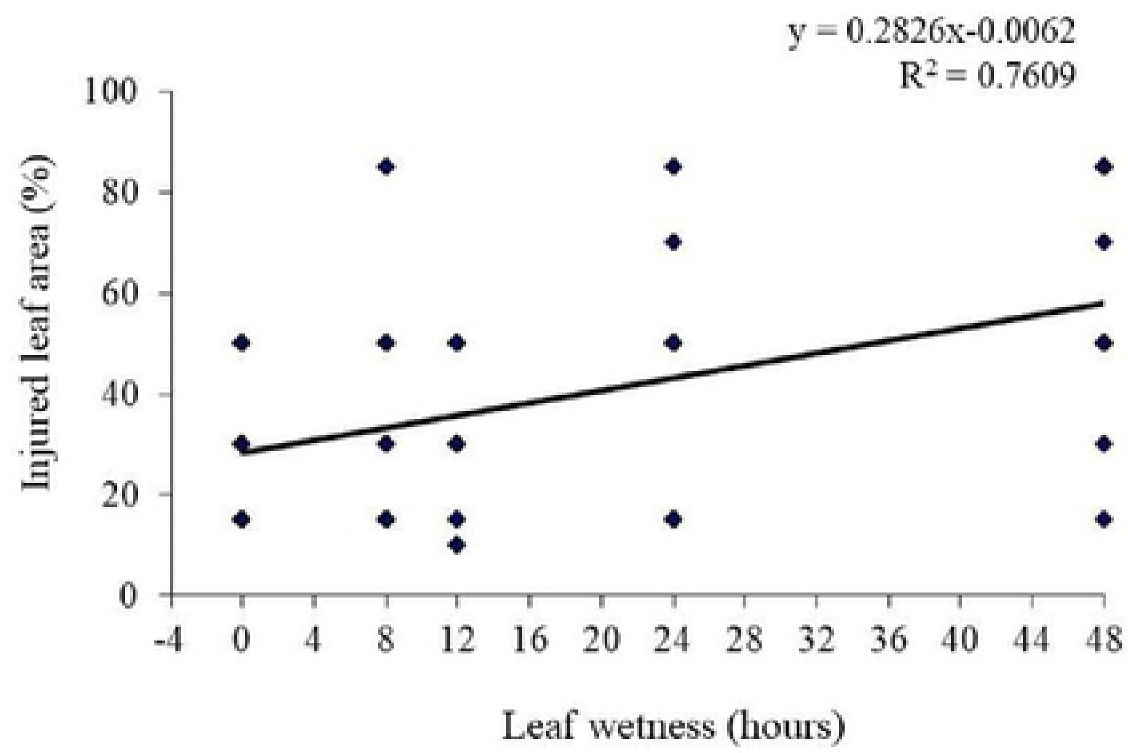


Figure 3 – Effect of leaf wetness on the severity of the desease caused by *C. typhae* in plants of *T. domingensis*, in the greenhouse.

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Fig 3