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Note

Survival of *Pochonia chlamydosporia* on the soil surface after different exposure intervals at ambient conditions

Rafael Henrique Fernandes[a], Everaldo Antônio Lopes[a,*], Darlan Ferreira Borges[a], Amanda Ferreira Bontempo[a], José Cola Zanuncio[b], José Eduardo Serrão[b]

[a] *Universidade Federal de Viçosa – Campus Rio Paranaíba (UFV-CRP), Instituto de Ciências Agrárias, Caixa Postal 22, Rio Paranaíba, Minas Gerais 38810-000, Brazil*
[b] *Universidade Federal de Viçosa – Campus Viçosa, Departamento de Entomologia, Viçosa, Minas Gerais 36570-900, Brazil*

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ABSTRACT

Background: Exposure of the nematophagous fungus *Pochonia chlamydosporia* to solar radiation and elevated temperatures before being incorporated into the soil can reduce its survival and efficiency as biocontrol agent.
Aims: A field experiment was carried out to assess the effect of the exposure period on the viability of *P. chlamydosporia* applied on the soil surface.
Methods: A commercial bionematicide based on *P. chlamydosporia* was sprayed on soil, and soil samples were collected before and at 0, 30, 60, 90, 120, and 150 min after fungal application. Relative humidity (RH), the irradiance (IR), air temperature (AT), and soil temperature (ST) were recorded. The number of *P. chlamydosporia* colony forming units (CFUs) was evaluated after 20 days of incubation.
Results: *P. chlamydosporia* survival decreased over the time of exposure on the soil surface. Overall, the number of CFUs decreased by more than 90% at 150 min after application. Exposure to RH ≥61%, ST and AT between 25–35 °C and 19–29 °C, and IR between 1172 and 2126 μmol of photons m^{-2} s^{-1} induced a negative exponential effect on the survival of the fungus over the time.
Conclusions: Exposure to climatic conditions on the soil surface reduces *P. chlamydosporia* viability.

Supervivencia de *Pochonia chlamydosporia* en la superficie del suelo después de diferentes intervalos de exposición a condiciones ambientales

Palabras clave:
Control biológico
Irradiación
Humedad relativa
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RESUMEN

Antecedentes: La exposición del hongo nematófago *Pochonia chlamydosporia* a la radiación solar y la temperatura elevada antes de ser incorporado al suelo puede reducir su supervivencia y eficiencia como agente de biocontrol.
Objetivos: Se realizó un experimento de campo para evaluar el efecto del período de exposición a condiciones ambientales sobre la viabilidad de *P. chlamydosporia* en la superficie del suelo.
Métodos: Se pulverizó sobre el suelo un bionematicida comercial hecho a base de *P. chlamydosporia* y se recogieron muestras de suelo antes y después de 0, 30, 60, 90, 120 y 150 min tras la aplicación del hongo. Se registraron la humedad relativa (HR), la irradiación (IR), la temperatura del aire (TA) y la temperatura del suelo (TS). Se evaluó el número de unidades formadoras de colonias (UFC) de *P. chlamydosporia* después de 20 días de incubación.
Resultados: La supervivencia de *P. chlamydosporia* disminuyó durante el tiempo de exposición en la superficie del suelo. En general, el número de UFC disminuyó en más de un 90% a los 150 min después de la aplicación. La exposición a HR ≥ 61%, TS y TA entre 25-35 °C y 19-29 °C, e IR entre 1.172 y 2.126 μmol de fotones m^{-2} s^{-1} indujo un efecto exponencial negativo en la supervivencia del hongo.
Conclusiones: La exposición a las condiciones climáticas en la superficie del suelo reduce la viabilidad de *P. chlamydosporia*.

* Corresponding author.
E-mail address: everaldolopes@ufv.br (E.A. Lopes).

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Pochonia chlamydosporia parasitizes phytonematodes.[12,23] It produces chlamydospores that enhance its establishment and survival in the soil.[12] Incorporating chlamydospores into the soil increases the probability of nematode egg parasitism and protects them from adverse surface environmental conditions.[3] In some cases, however, *P. chlamydosporia*-based bionematicides are applied on the soil surface[3] and knowledge is scarce about the effect on this fungus of high temperatures and solar radiation.

We hypothesize that temperatures higher than the optimal range of 24–28 °C[1] and extended exposure to ultraviolet radiation reduce *P. chlamydosporia* viability, as reported for other fungi,[4,15,18] despite producing chlamydospores. We assessed *P. chlamydosporia* viability after exposure on the soil surface for 0, 30, 60, 90, 120, and 150 min.

We carried out a field experiment at the UFV-CRP. *P. chlamydosporia* isolate Pc-10 was used as a wettable pow-

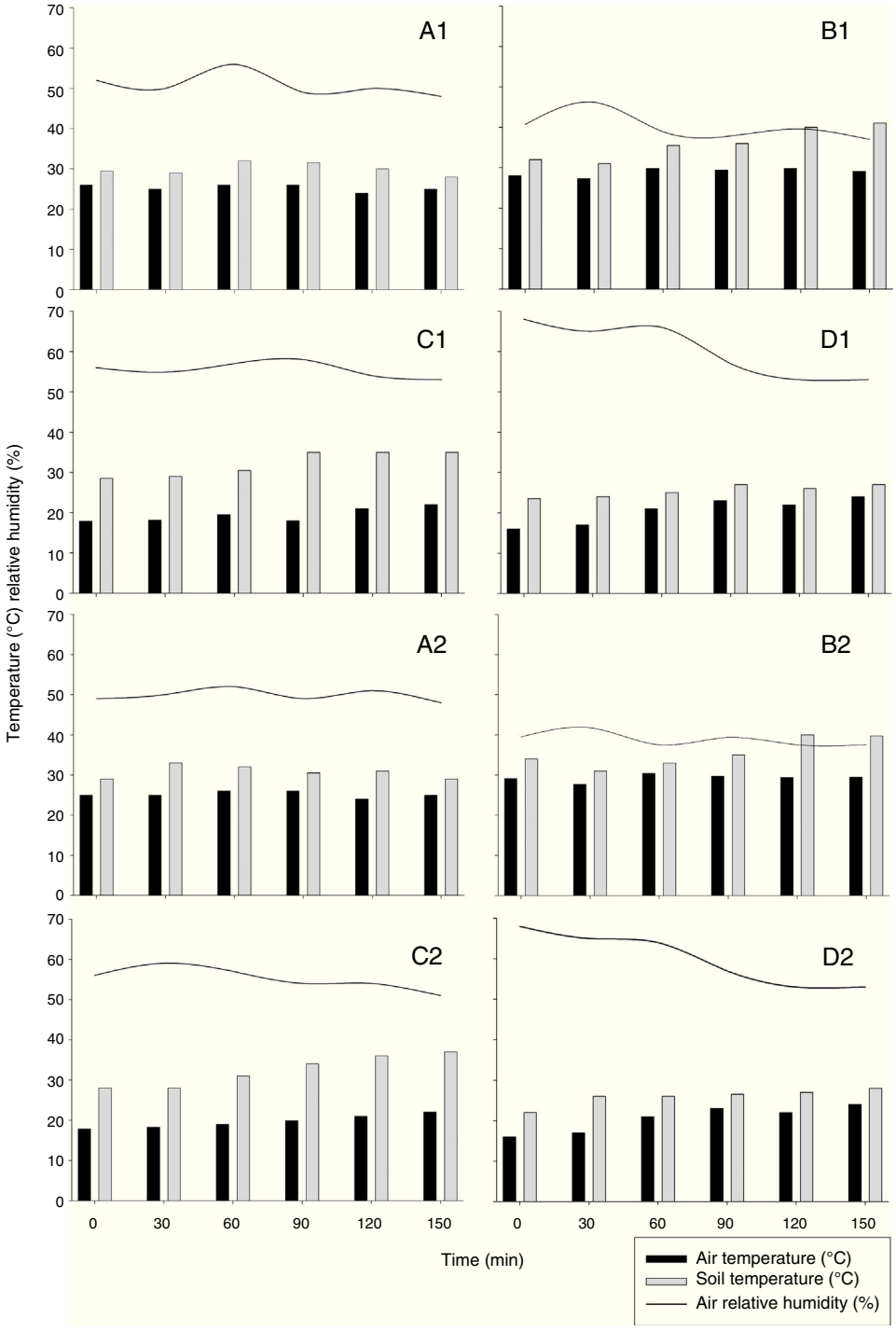

Fig. 1. Air and soil temperature (°C) and relative humidity (%) at sampling time after the application of *Pochonia chlamydosporia* var. *chlamydosporia* isolate Pc-10 on the soil surface. Replica 1: 1st week (A1); 2nd week (B1); 3rd week (C1); 4th week (D1). Replica 2: 1st week (A2); 2nd week (B2); 3rd week (C2); 4th week (D2).

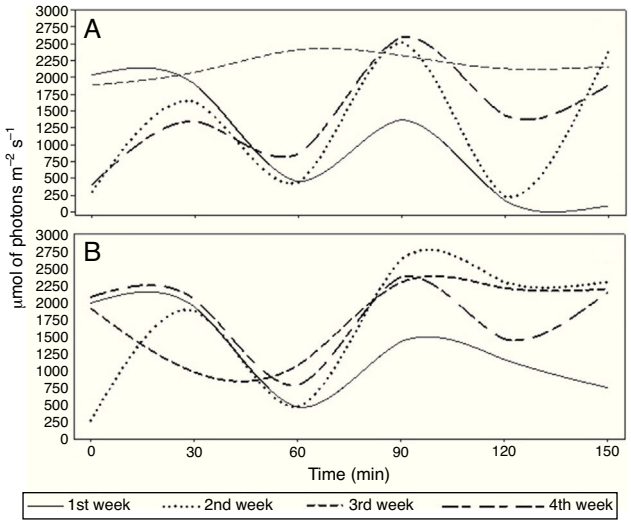

Fig. 2. Solar irradiance at sampling time after the application of *Pochonia chlamydosporia* var. *chlamydosporia* isolate Pc-10 on the soil surface in the experiment (Replica 1 – A; Replica 2 – B).

der bionematicide (Rizotec®, Rizoflora Biotecnologia, 3×10^8 viable chlamydospores/g). We diluted and applied Pc-10 on raised beds (1.6 m wide and 0.30 high) on the soil surface using a backpack sprayer pressurized with CO_2, with spray volume of 300 l/ha. We applied the bionematicide between 01:00 and 03:30 pm. We collected soil samples before and at 0, 30, 60, 90, 120, and 150 min after application. We collected five soil samples per plot (2.5 m length and 1.6 m wide) with 50 mm diameter PVC pipe inserted 5 cm deep in the soil. We bulked the samples to form a composite sample and stored them at 4 °C until use. We recorded relative humidity (RH, %), air temperature (AT, °C), soil temperature (ST, °C) (Fig. 1) and irradiance (IR, µmol of photons m^{-2} s^{-1}) (Fig. 2) at the time of soil sampling.

We repeated the applications for four weeks. The experiment was performed twice simultaneously in different locations of the field, using similar protocols. Experimental replicas will hereafter be referred to as replicas 1 and 2. We used a randomized block design with seven periods after *P. chlamydosporia* application and four weeks of evaluations (blocks). In both the replicas, average RH, AT, ST and IR ranged from 39.14 to 61.28%, 19.21 to 29.17 °C, 25.07 to 35.36 °C and 1172 to 2126 µmol of photons m^{-2} s^{-1}, respectively (Figs. 1 and 2). We assessed RH, IR, and AT using infrared gas analyzer (IRGA Li-Cor 6400 XT with integrated fluorescence) and recorded ST using thermometers buried 5 m deep into the soil. Average soil moisture (SM), determined by the gravimetric method,[2] was 16.4, 6.3, 19.2 and 20% at weeks 1, 2, 3, and 4, for both replicas. We assessed the viability of *P. chlamydosporia* by using colony-forming units (CFUs) assay,[9] plating soil suspension onto semi-selective medium.[8]

We tested data for normality (Kolmogorov–Smirnov test) and homoscedasticity (Bartlett test). Regression analysis ($p < 0.05$) was used to study the effect of *P. chlamydosporia* exposure periods on the soil over the number of CFUs. We used R version 3.1.1 for statistical analyses.[16]

P. chlamydosporia survival was reduced exponentially or linearly over the time of exposure on the soil surface (Fig. 3). We did not recover any *P. chlamydosporia* colony from samples collected before *P. chlamydosporia* application. Therefore, CFUs recovered in the experiment were not due to the presence of native isolates. The number of *P. chlamydosporia* CFUs decreased by more than 90% at 150 min after application, except in the third week after *P. chlamydosporia* surface soil application in the replica 1 (Fig. 3 C1). In the first week, the number of CFUs decrease rate (DR) was approximately 46% and 64% at every 30 min of exposure in replicas 1 and 2 (Figs. 3 A1 and A2). RH, ST, AT and IR ranged 50–50.8%, 30–30.6 °C, 25–25.1 °C, and 1172–1378 µmol of photons m^{-2} s^{-1} (Figs. 1 and 2).

P. chlamydosporia viability was reduced by 55 and 69% at every 30 min in the second week (Figs. 3 B1 and B2), when RH, ST, AT and IR ranged 39.1–40.5%, 35.2–35.4 °C, 28.8–29.2 °C, and 1630–1638 µmol of photons m^{-2} s^{-1}. Fungus survival was higher in the third week, especially in replica 1 (Fig. 3 C1), with 19% DR at every 30 min. RH, ST, AT and IR ranged 54.2–55.1%, 31.4–31.9 °C, 19.2–19.3 °C, and 1593–2126 µmol of photons m^{-2} s^{-1} (Figs. 1 and 2). In the fourth week, *P. chlamydosporia* viability decreased by 38 and 51% at every 30 min of exposure (Figs. 3 D1 and D2).

ST and AT between 25–35 °C and 19–29 °C, IR between 1172 and 2126 µmol of photons m^{-2} s^{-1} and RH ≥61% induced a negative exponential effect on *P. chlamydosporia* survival over time, which may reduce its ability to control nematodes.

High and low temperatures limit the survival and growth of soil fungi, especially <5 °C and >30 °C.[22] *P. chlamydosporia* optimal range is 24–28 °C,[1] although temperature tolerance may vary according to the fungus strain origin. AT in the first two weeks was around 25 °C, reaching 30 °C, whereas ST was around 30 °C and reached 40 °C. A suitable *P. chlamydosporia* temperature range (20–30 °C) allowed higher survival rate in the third and fourth weeks, especially until 60 min after application. Low water availability (LWA) in the soil also reduces *P. chlamydosporia* survival.[19] SM and RH in the first two weeks ranged 6.3–16.3% and 40–57%, whereas they ranged 19.2–20% and 55–69% in the third and fourth weeks.

Acting synergistically with high temperatures and LWA, the cumulative effect of the exposure to solar radiation reduced *P. chlamydosporia* viability, as reported for other biocontrol agents.[4,11,13,14] UV-A and UV-B rays cause genetic and morphological damage in fungi,[17,20] reducing the longevity of the propagules.[6,7,14] Melanin protects fungi against radiation.[10,21] *P. chlamydosporia* chlamydospores do not contain melanin,[24] making them more vulnerable to radiation. IR levels were always above 1000 µmol of photons m^{-2} s^{-1} at 30 min of exposure and reached 2080 µmol of photons m^{-2} s^{-1} in the third week of replica 1. To enhance nematode control, *P. chlamydosporia*-based bionematicides must be incorporated into the soil[3] or the formulation must have compounds for protecting the fungus from adverse environmental conditions,[5] such as high temperatures (>25 °C) and high irradiance (>1000 µmol of photons m^{-2} s^{-1}).

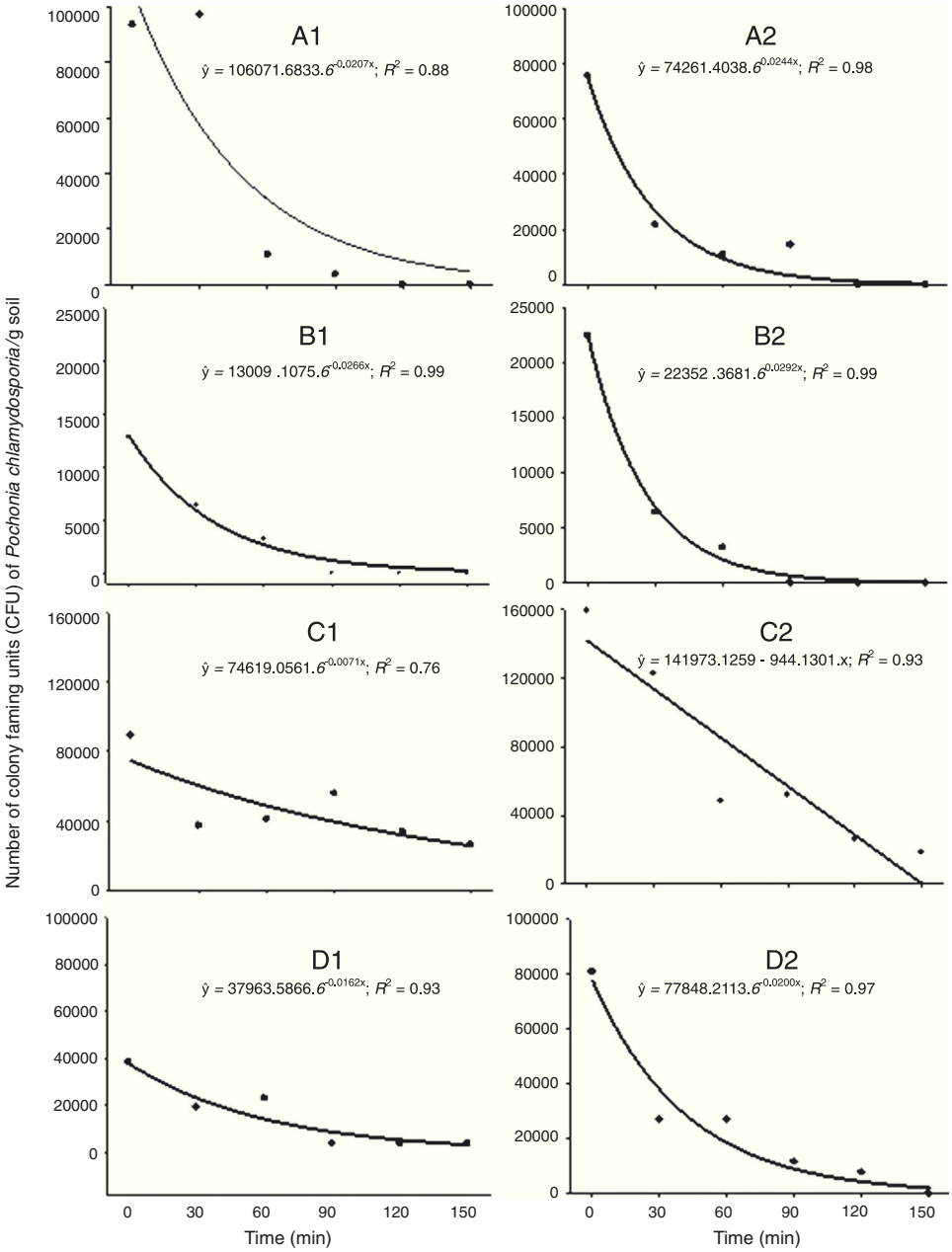

Fig. 3. Number of colony forming units (CFU/g of soil) of *Pochonia chlamydosporia* var. *chlamydosporia* isolate Pc-10 recovered from soil samples collected at different periods after the application of the fungus on the surface of the seed beds. Replica 1: 1st week (A1); 2nd week (B1); 3rd week (C1); 4th week (D1). Replica 2: 1st week (A2); 2nd week (B2); 3rd week (C2); 4th week (D2).

Conflict of interest

The authors have no conflict of interest to declare.

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References

1. Arevalo J, Hidalgo-Diaz L, Martins I, Souza JF, Castro JMC, Carneiro RMDG, et al. Cultural and morphological characterization of *Pochonia chlamydosporia* and *Lecanicillium psalliotae* isolated from *Meloidogyne mayaguensis* eggs in Brazil. Trop Plant Pathol. 2009;34:158–63, http://dx.doi.org/10.1590/S1982-56762009000300004
2. Bernardo S, Soares AA, Mantovani EC. Manual de irrigação. Viçosa: Editora UFV; 2006.
3. Bontempo AF, Fernandes RH, Lopes J, Freitas LG, Lopes EA. *Pochonia chlamydosporia* controls *Meloidogyne incognita* on carrot. Australas. Plant Pathol. 2014;43:421–4, http://dx.doi.org/10.1007/s13313-014-0283-x
4. Braga GUL, Rangel DEN, Flint SD, Miller CD, Anderson AJ, Roberts DW. Damage and recovery from UV-B exposure in conidia of the entomopathogens *Verticillium lecanii* and *Aphanocladium album*. Mycologia. 2004;94:912–20, http://dx.doi.org/10.2307/3761859
5. Burges HD. Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatments. Dordrecht: Kluwer Academic Publishers; 1998.
6. Costa LB, Rangel DEN, Morandi MAB, Bettiol W. Effects of UV-B radiation on the antagonistic ability of *Clonostachys rosea* to *Botrytis cinerea* on strawberry leaves. Biol Control. 2013;65:95–100, http://dx.doi.org/10.1016/j.biocontrol.2012.12.007
7. Costa LB, Rangel DEN, Morandi MAB, Bettiol W. Impact of UV-B radiation on *Clonostachys rosea* germination and growth. World J Microbiol Biotechnol. 2012;28:2497–504, http://dx.doi.org/10.1007/s11274-012-1057-7
8. Gaspard T, Jaffee BA, Ferris H. Association of *Verticillium chlamydosporium* and *Paecilomyces lilacinus* with root-knot nematode infested soil. J Nematol. 1990;22:207–13.

9. Kerry BR, Bourne JM. A Manual for research on *Verticillium chlamydosporium*, a potential biological control agent for root-knot nematodes. Gent: IOBC-WPRS; 2002.
10. Kunwar A, Adhikary B, Jayakumar S, Barik A, Chattopadhyay S, Marmentini GA, et al. Melanin, a promising radioprotector: mechanisms of actions in a mice model. Toxicol Appl Pharmacol. 2012;264:202–11, http://dx.doi.org/10.1016/j.taap.2012.08.002
11. Li J, Feng MG. Intraspecific tolerance of *Metarhizium anisopliae* conidia to the upper thermal limits of summer with a description of a quantitative assay system. Mycol Res. 2009;113:93–9, http://dx.doi.org/10.1016/j.mycres.2008.08.006
12. Manzanilla-Lopez RH, Esteves I, Finetti-Sialer M, Hirsch P, Ward E, Devonshire J, et al. *Pochonia chlamydosporia*: advances and challenges to improve its performance as a biological control agent of sedentary endo-parasitic nematodes. J Nematol. 2013;45:1–7.
13. Morandi MAB, Maffia LA, Mizubuti ESG, Alfenas AC, Barbosa JG, Cruz CD. Relationships of microclimatic variables to colonization of rose debris by *Botrytis cinerea* and the biocontrol agent *Clonostachys rosea*. Biocontrol Sci Technol. 2006;16:619–30, http://dx.doi.org/10.1080/09583150600699739
14. Morandi MAB, Mattos LPV, Santos ER, Bonugli RC. Influence of application time on the establishment, survival, and ability of *Clonostachys rosea* to suppress *Botrytis cinerea* sporulation on rose debris. Crop Prot. 2008;27:77–83, http://dx.doi.org/10.1016/j.cropro.2007.04.008
15. Nascimento E, Silva SH, Marques ER, Roberts DW, Braga GUL. Quantification of cyclobutane pyrimidine dimers induced by UVB radiation in conidia of the fungi *Aspergillus fumigatus*, *Aspergillus nidulans, Metarhizium acridum* and *Metarhizium robertsii*. Photochem Photobiol. 2010;86:1259–66, http://dx.doi.org/10.1111/j.1751-1097.2010.00793.x
16. R Development Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2014. Available from: http://R-project.org [accessed 15.12.14].
17. Rangel DEN, Butler MJ, Torabinejad J, Anderson AJ, Braga GUL, Day AW, et al. Mutants and isolates of *Metarhizium anisopliae* are diverse in their relationships between conidial pigmentation and stress tolerance. J Invertebr Pathol. 2006;93:170–82, http://dx.doi.org/10.1016/j.jip.2006.06.008
18. Rangel DEN, Fernandes EKK, Braga GUL, Roberts DW. Visible light during mycelial growth and condition of *Metarhizium robertsii* produces conidia with increased stress tolerance. FEMS Microbiol Lett. 2011;315:81–6, http://dx.doi.org/10.1111/j.1574-6968.2010.02168.x
19. Santos MCV, Esteves I, Abrantes I. In vitro water stress bioassays with the nematophagous fungus *Pochonia chlamydosporia*: effects on growth and parasitism. Biol Control. 2012;63:310–9, http://dx.doi.org/10.1016/j.biocontrol.2012.09.001
20. Santos MP, Dias LP, Ferreira PC, Pasin LAAP, Rangel DEN. Cold activity and tolerance of the entomopathogenic fungus *Tolypocladium* spp. to UV-B irradiation and heat. J Invertebr Pathol. 2011;108:209–13, http://dx.doi.org/10.1016/j.jip.2011.09.001
21. Valero A, Begum M, Leong SL, Hocking AD, Ramos AJ, Sanchis V, et al. Effect of germicidal UVC light on fungi isolated from grapes and raisins. Lett Appl Microbiol. 2007;45:238–43, http://dx.doi.org/10.1111/j.1472-765X.2007.02175.x
22. Van Damme V, Hoedekie A, Viane N. Long-term efficacy of *Pochonia chlamydosporia* for management of *Meloidogyne javanica* in greenhouse crops. Nematology. 2005;7:726–36.
23. Viggiano JR, Freitas LG, Lopes EA. Use of *Pochonia chlamydosporia* to control *Meloidogyne javanica* in cucumber. Biol Control. 2014;69:72–7, http://dx.doi.org/10.1016/j.biocontrol.2013.11.004
24. Zare R, Gams W, Evans HC. A revision of *Verticillium* section Prostata. V. The genus *Pochonia*, with notes on *Rotiferophthora*. Nova Hedwigia. 2001;73:51–86.