LOUREIRO, E. S.; PESSOA, L. G. A.; DIAS, P. M.; RIBEIRO, M. P.; TOSTA, R. A. S.; TEODORO, P. E. Hydration levels on conidial production of *Metarhizium rileyi* (Ascomycota) in solid growing medium. **Revista de Agricultura Neotropical**, Cassilândia-MS, v. 6, n. 3, p. 48-52, jul./set. 2019. ISSN 2358-6303.

Revista de Agricultura Neotropical

Hydration levels on conidial production of *Metarhizium rileyi* (Ascomycota) in solid growing medium

Elisângela de Souza Loureiro[1], Luis Gustavo Amorim Pessoa[1], Pamella Mingotti Dias[2], Muller De Paula Ribeiro[1], Ricardo Alexandre de Souza Tosta[1], Paulo Eduardo Teodoro[1]

[1] Universidade Federal de Mato Grosso do Sul, Campus de Chapadão do Sul, Chapadão do Sul, Mato Grosso do Sul, Brasil. E-mail: elisangela.loureiro@ufms.br, luis.pessoa@ufms.br, mullerdepaularibeiro@hotmail.com, ricardoagronomia2014@gmail.com, eduteodoro@hotmail.com

[2] Universidade Federal da Grande Dourados, Dourados, Mato Grosso do Sul, Brasil. E-mail: pamellamingotti@hotmail.com

Received: 08/08/2018; Accepted: 30/05/2019.

ABSTRACT

This study aimed to evaluate the conidial production of *Metarhizium rileyi* in rice with different water volumes. The bioassay was composed by completely randomized design (CRD), with four treatments (20, 30, 40 and 50 mL of distilled water), being added 100 g of rice thin and long, making a total of 10 plastic bags per treatment, which were autoclaved for 15 minutes at 1.0 atm pressure, to 120 ºC. After the cooling of the rice, were added in each plastic bag, 2.0 mL of suspension containing 1×10^8 conidia mL^{-1}. Then the bags were incubated for ten days in a germination chamber (BOD type) at 25 °C (±1 °C), 80% (±10%) relative humidity and 12h photoperiod to promote conidial germination and growth of the fungus, being performed a mild agitation every two days. The use of higher water volume resulted in greater conidial production and greater number of viable conidia. However, the highest rate of conidia germination was obtained with the use of 30 mL of water, is this the volume of water that corresponds to the best results.

Keywords: entomopathogenic fungus, microbial control, substrate for production of microorganisms.

Níveis de hidratação na produção de *Metarhizium rileyi* (Ascomycota) em meio sólido

RESUMO

Objetivou-se estudar a produção de *Metarhizium rileyi* em arroz com diferentes concentrações de água. O bioensaio foi composto por delineamento inteiramente casualizado (DIC), com quatro tratamentos (20, 30, 40 e 50 mL de água destilada), sendo adicionados 100 g de arroz fino e longo, perfazendo um total de 10 sacos plásticos por tratamento, os quais foram autoclavados por 15 minutos a 1 atm de pressão, a 120 ºC. Após o resfriamento do arroz, foram adicionados em cada saco plástico 2 mL da suspensão contendo 1×10^8 conídios mL^{-1}. Em seguida, os sacos foram acondicionados por 10 dias em câmara climatizada tipo B.O.D. (a 25±1 ºC, 80±10% UR e fotofase de 12h) para promover germinação dos conídios e crescimento do fungo, sendo realizada uma leve agitação a cada dois dias. Verificou-se que a maior dose de água proporcionou maior produção de conídios e maior número de conídios viáveis. Contudo, para a germinação, o ponto de máximo é aproximadamente 30 mL, sendo essa a dose que proporcionou melhor resultado.

Palavras-chave: fungo entomopatogênico, controle microbiano, substrato de produção de microrganismos.

1. Introduction

The indiscriminate use of chemical insecticides for the control of several pests has increased the population of insects resistant to certain molecules, in addition to the emergence of new pest insects that were previously considered as secondary pests (Méndez et al., 2010). The fact that these insecticides are not completely efficient in controlling pests, interfering in the success of Integrated Pest Management (IPM), has provided a series of economic losses, besides the imbalance and disruption of ecosystems (Céspedes et al., 2008).

Based on the transition of modern agriculture for sustainable agriculture, the use of biological control is economically and ecologically viable alternative in the control of pests (Perinotto et al., 2012). This fact has been observed in recent decades, with the growing use of entomopathogenic fungi (Faria and Wraight, 2007), due to some aspects such as low cost, high efficiency, less impact on beneficial organisms, reduction of waste on the environment and increasing the biodiversity of ecosystems, when compared with the chemical insecticides (Lacey et al., 2001).

Among the entomopathogenic fungi, *Nomuraea rileyi*, reclassified as *Metarhizium rileyi* (Ascomycota: Clavicipitaceae) (Kepler et al., 2014), is regarded as one of the main causative agents of natural mortality of several Lepidoptera species in wide range of ecosystems and crops (Ignoffo, 1981; Chaudhari et al., 2015). In the last two decades, this species has been intensively investigated in different geographic locations, being reported great variability in virulence and specificity in function of different and narrow range of hosts (Sujii et al., 2002; Devi et al., 2003; Chen et al., 2012; Song et al., 2016).

M. rileyi is a dimorphic fungus entomopathogenic fungus, with growth in culture medium is characterized by an initial stage yeast (similar to the bacteria culture), which will slowly turning into mycelium (Suwannakut et al., 2005) and does not have a pH saprophytic colonization in the soil (Boucias et al., 2000). This fungus is demanding in terms of nutrition, particularly about the source of carbon, nitrogen, as well as good aeration and humidity for adequate sporulation (Devi et al., 2003), and germ tube growth (Boucias et al., 2000).

The conidia are the structures produced on the surface of a solid growing medium, within different containers, depending on the purpose and scale of production (Almeida and Batista Filho, 2006). Its production in a solid substrate, when compared to the fermentation liquid, does not provide good yields of these structures, requiring tedious sporulation conditions along with stimulating light, which ends up limiting the fermentation in the earth and its subsequent marketing (Faria and Wraight, 2007).

The production techniques of fungi for the control of pests must have low cost and enable the achievement of a high concentration of viable and virulent forms of the pathogen, which can be formulated and used (Loureiro et al., 2005). Natural substrates, solids, such as rice and wheat bran, constitute appropriate culture media for the massive conidial production (Méndez et al., 2009), due to the fact of providing physical conditions such as size, grain shape, suitable surface properties of hydration, aeration and structural integrity even after colonization by fungus (Jenkins et al., 1998; Bhanu Prakash et al., 2008), in addition to the adequate nutritional balance (Sahayaraj and Karthick, 2008).

Méndez et al. (2009) and Bhanu Prakash et al. (2008) stated that the water volume of the solid substrate is determinant in the income of the conidia of mitosporic fungi, being associated with the increased availability of oxygen. According to Devi et al. (2001), the availability of this element and indispensable for the sporulation of *M. rileyi*. Therefore, there is a need to study different water volumes to maximize the production of this species of fungus in a solid medium. As there is no information on the production of *M. rileyi* entomopathogenic fungus in a solid medium, the objective of this work was to study the production of this fungus in rice with the use of different water volumes.

2. Material and Methods

In vitro assays were performed, using the isolated from *M. rileyi* UFMS 03 (isolated from *Alabama argillacea* Hübner (Lepidoptera: Noctuidae) multiplied in Petri plates containing Sabouraud Dextrose Agar (SDA) medium. Subsequently, these plates were incubated for ten days in a germination chamber (BOD type) at 25 °C (±1 °C), 80% (±10%) relative humidity and 12h photoperiod to promote the growth and sporulation of the fungus. After this period, the conidia were removed by scraping with metal handle and then prepared the suspension containing 1×10^8 conidia mL^{-1} with sterile distilled water and spreader decal (Tween 80®) to 0.1%.

The bioassay was composed by completely randomized design (CRD), with four treatments (20, 30, 40 and 50 mL of distilled water) and ten replicates, each one composed of plastic bag. In each plastic bag (35 × 22 cm) was added 100 g of rice, thin and long (type 1) and 20, 30, 40 and 50 mL of sterile distilled water, respectively, closed later with metal staples and autoclaved for 15 minutes at 1.0 atm pressure, to 120 ºC. After the autoclaving, the bags were packed inside of laminar flow until the complete cooling (Loureiro et al., 2005).

After the cooling of the rice, were added in each plastic bag, 2.0 mL of suspension containing 1 × 10^8 conidia mL^{-1}. The inoculation was performed with the aid of a pipette, automatic closing then the container with metal staples and stirring the contents in order to standardize the distribution of inoculum. Then the bags were stored for 10 days in a germination chamber (BOD type) at 25 °C (±1 °C), 80% (±10%) relative humidity and 12h photoperiod to promote the conidial germination and mycelial growth of the fungus, being performed a mild agitation every two days, according to the methodology adapted by Freitas et al. (2014).

For the determination of the conidia production in each treatment, it was removed, at random, 1.0 g of rice containing the fungus of each repetition, adding to it 10 mL of sterile distilled water more spreader decal (Tween 80®) to 0.1%. Then, the samples were diluted in series and quantified in a Neubauer chamber with the aid of an optical microscope, with an increase of 400 x (Alves and Pereira, 1998).

For the reading of the conidial germination, a random sample of 1.0 g of rice with fungus for each plastic bag was removed, and the suspension was placed into three Petri dishes containing Potato Dextrose Agar (PDA) medium, for the different treatments.

The plates were incubated for 20 hours at 26 °C (±1 °C), 80% (±10%) relative humidity, and 12 h photoperiod. Then, to quantify the number of germinated and ungerminated conidia of the quadrants in the optical microscope, with an increase of 400 x (Alves et al., 2008).

For the analysis of associations between water volumes and conidia germination, the productivity and viable conidia g^{-1} of the substrate, were employed the regression analysis and analysis of canonical variables (ACV) using the Rbio software (Bhering, 2017).

3. Results and Discussion

The higher water volume in the growing medium provided greater conidial production and greater number of viable conidia (Figures 1A and 1C). However, the higher conidia germination rate was obtained with the use of 30 mL of water (Figure 1B).

These results are reinforced by multivariate analysis of canonical variables, which demonstrated that treatment with 30 mL provided both higher germination (germ) and production of conidia (Prod) (Figure 2).

The results obtained in this study can be associated with a greater volume of water in the culture medium. According to Méndez et al. (2009), this is one of the parameters that determine the income of conidia of mitosporic fungi. This effect is directly related to the increase of oxygen availability (Bhanu Prakash et al., 2008), the latter being an indispensable element for the sporulation of *M. rileyi* (Devi et al., 2001).

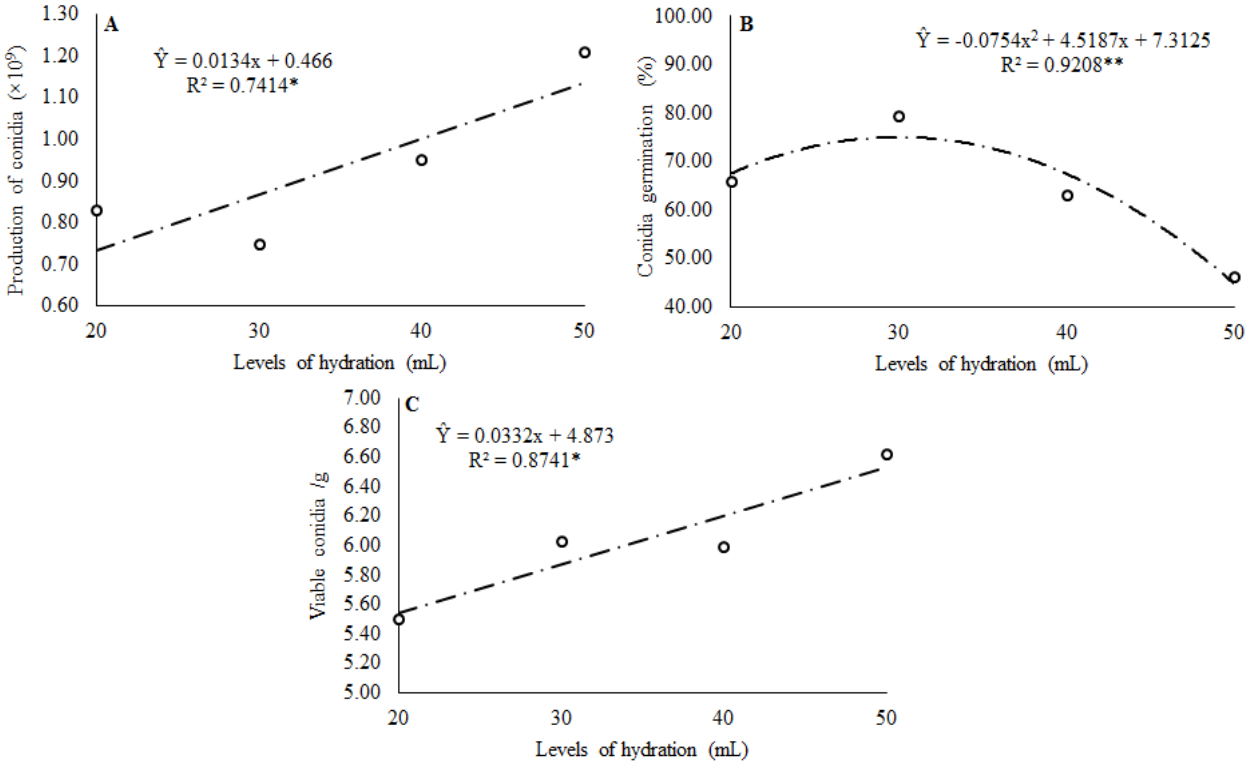

Figure 1. Effects of water volumes in the growing medium on conidial production (A) and conidia germination (B) and viable conidia (C) of *Metarhizium rileyi*.

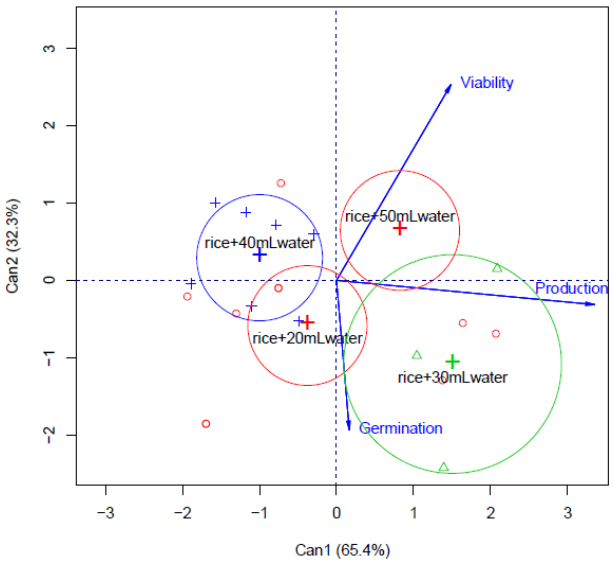

Figure 2. Canonical analysis for the different water volumes about the conidial production in rice, conidia germination, and viability (g) of *Metarhizium rileyi* (25±1 °C, 80±10% relative humidity and 12h photoperiod).

The higher water volume in the culture medium does not always result in higher rates of conidia germination (Figure 1B). According to Song et al. (2016), this fact occurs due to a blockage of the interstitial pores, which consequently results in the fall of gas exchange, compromising the microbial metabolism. In this aspect, the addition of 30 mL water resulted in higher conidia germination percentage than the treatment with the addition of 50 mL.

The development of the fungus requires adequate availability of water in the growth medium, favoring the transport of nutrients and metabolites in dissolved form, also, to maintain the cell volume to connect with molecules of poliois, sugars and enzymes (Crowe et al., 1982).

New studies using different volumes of water and isolated are required to clarify the potential of this agent better, increasing production and germination of conidia in less time, to propose a technique of production of *M. rileyi* economically viable.

4. Conclusions

The use of 30 mL of water in the growing medium was the most efficient in the germination and production of viable conidia. The production of isolated *M. rileyi* UFMS 03 in rice was more efficient with the water volume of 30 mL because it increased the germination rate of the fungus.

Bibliographic References

Alves, S.B., Lopes, R.B., Vieira, S.A., Tamai, M.A., 2008. Fungos entomopatogênicos usados no controle de pragas na América Latina, in: Alves, S.B., Lopes, R.B., (Ed.), Controle microbiano de pragas na América Latina. FEALQ, Piracicaba, p. 69-110.

Alves, S.B., Pereira, R.M., 1998. Produção de fungos entomopatogênicos, in: Alves, S.B., Lopes, R.B., (Ed.), Controle microbiano de insetos. FEALQ, Piracicaba, p. 845-870.

Almeida, J.E.M., Batista Filho, A.B., 2006. Microorganismos no controle de pragas, in: Pinto, A.S., Nava, D.E., Rossi, M.M., Malerbo-Souza, D.T., (Ed.), Controle Biológico de pragas: na prática. CP2. Piracicaba, p. 35-44.

Bhanu Prakash, G.V., Padmaja, V., Siva Kiran, R.R., 2008. Statistical optimization of process variables for the large-scale production of *Metarhizium anisopliae*. Bioresource Technology, 99(6), 1530-1537.

Bhering, L.L., 2017. Rbio: A Tool For Biometric And Statistical Analysis Using The R Platform. Crop Breeding and Applied Biotechnology, 17, 187-190.

Boucias, D.G., Stokes, C., Suazo, A., Funderbuck, J., 2000. AFLP analysis of the entomopathogen *Nomuraea rileyi*. Mycologia, 92(4), 638-648.

Céspedes, I., Del Pozo, E., Garcia, I., Méndez, A., 2008. Efecto de la temperatura sobre el hongo entomopatógeno *Nomuraea rileyi* (Farlow) Samson y su efectividad sobre *Spodoptera frugiperda* J. E. Smith. Revista Protección Vegetal, 23(3), 176-182.

Chaudhari, C.S., Chandele, A.G., Pokharkar, D.S., Dethe, M.D., Firake, D.M., 2015. Pathogenicity of Different Isolates of Entomopathogenic Fungus, *Nomuraea rileyi* (Farlow) Samson Against Tobacco Caterpillar, *Spodoptera litura* (Fabricius). Proceedings of the National Academy of Sciences, 2, 1-7.

Chen, H., Yin, Y.P., Li, Y., Mahmud, M.S., Wang, Z.K., 2012. Identification and analysis of genes differentially expressed in the *Spodoptera litura* fat body in response to the biocontrol fungus, *Nomuraea rileyi*. Comparative Biochemistry and Physiology, 163, 203-210.

Crowe, J.H., Crowe, L.M., Deamer, D.W., 1982. Hydration dependent phase changes in biological membrane, in: Biophysics of Water, F. Fanks & S. Mathias, (Ed.), Wiley, Chichester, p. 295-299.

Devi, P.S.V., Prasad, Y.G., Chowdary, D.A., Rao, L.M., Balakrishnan, K., 2003. Identification of virulent isolates of the entomopathogenic fungus *Nomuraea rileyi* (F) Samson for the management of *Helicoverpa armigera* and *Spodoptera litura.* Mycopathologia, 156(4), 365-373.

Devi, P.S., Chowdary, A., Prasad, Y.G., 2001. Cost-effective multiplication of the entomopathogenic fungus *Nomuraea rileyi* (F) Samson. Mycopathologia, 151(1), 35-39.

Faria, M., Wraight, S.P., 2007. Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biological Control, 43, 237-256.

Freitas, A.F., Loureiro, E.S., Almeida, M.E.B., Pessoa, L.G.A., 2014. Rendimento de conídios e germinação de

diferentes isolados de *Metarhizium anisopliae* (Metsch.) Sorok. (Ascomycota: Clavicipitaceae) cultivados em arroz. Arquivos do Instituto Biológico, 81(1), 75-78.

Ignoffo, C.M., 1981. The fungus *Nomuraea rileyi* as a microbial insecticide, in: Burges, H.D., (Ed.), Microbial control of pests and plants diseases 1970-1980. Academic Press, p. 413-538.

Jenkins, N.E., Heviofo, G., Langewald, J., 1998. Development of mass production technology for aerial conidia for use as mycopesticides. Biocontrol News and Information, 31(2), 21-31.

Kepler, R.M., Humber, R.A., Bischoff, J.F., Rehner, S.A., 2014. Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. Mycologia, 106, 464-480.

Lacey, L.A., Frutos, R., Kaya, H.K., Vail, P., 2001. Insect pathogens as biological control agents: Do they have a future? Biological Control, 2(3), 230-248.

Loureiro, E.S., Batista Filho, A., Almeida, J.E.M., Pessoa, L.G.A., 2005. Produção de isolados de *Metarhizium anisopliae* selecionados para o controle de *Mahanarva fimbriolata* (Stal, 1854). Arquivos do Instituto Biológico, 72(4), 469-472.

Méndez, A., Del Pozo, E., Garcia, I., 2009. Producción masiva de *Nomuraea rileyi* (Farlow) Samson mediante una alternativa de cultivo bifásico. Revista Protección Vegetal, 24(3), 156-161.

Méndez, A., Del Pozo, E., Garcia, I., González, A., 2010. Evaluación de sustratos sólidos para la producción masiva de *Nomuraea rileyi* (Farlow) Samson. Revista Protección Vegetal, 25(2), 108-112.

Perinotto, W.M.S., Terra, A.L.M., Angelo, I.C., Fernandes, E.K.K., Golo, P.S., Camargo, M.G., Bittencourt, V.R.E.P., 2012. *Nomuraea rileyi* as biological contro agents of *Rhipicephalus microplus* tick. Parasitology Research, 111(4), 1743-1748.

Sahayaraj, K., Karthick, S., 2008. Mass production of entomopathogenic fungi using agricultural products and byproducts. African Journal Biotechnology, 7(12), 1907-1910.

Song, Z.Y., Jiang, W., Yin, Y.P., Wang, Z.K., 2016. Polarity proteins Mrcdc24 and Mrbem1 required for hypha growth and microsclerotia formation in *Metarhizium rileyi*. Biocontrol Science Technology, 26, 733-745.

Sujii, E.R., Carvalho, V.A., Tigano, M.S., 2002. Cinética da esporulação e viabilidade de conídios de *Nomuraea rileyi* (Farlow) Samson sobre cadáveres da lagarta-da-soja, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), em condições de campo. Neotropical Entomology, 31, 85-90.

Suwannakut, S., Boucias, D.G., Wiwat, E.C., 2005. Genotypic analysis of *Nomuraea rileyi* collected from various noctuid hosts. Journal of Invertebrate Pathology, 90(3), 169-176.