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Article

Solid fermentation of *Metarhizium robertsii*: substrate and culture conditions on conidia production and the biological efficacy

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Abstract

The implementation of entomopathogenic fungal conidia represents an alternative with advantages compared to chemical insecticides. Their production can be carried out on inexpensive substrates. The culture conditions, including substrates, temperature, moisture, and aeration rate for the production of Mr Xoch8.1 conidia in solid culture were examined; the research was conducted in Ecatepec de Morelos, State of Mexico, in 2022. An analysis of the quality of the conidia was carried out at the laboratory level, considering germination, viability, and infectivity. A conidiation profile was made during 11 days of culture of the Mr Xoch8.1 strain in solid fermentation. The highest production of conidia was observed at eight days. Production was evaluated on different substrates, with the production with white rice standing out. The incubation temperature at 28 °C was the most appropriate. The absence of forced aeration generated the highest production. The initial moisture of 60% led to a high production of conidia. The germination and viability of the conidia were 93% and 57%, respectively. In terms of infectivity, a mortality of 60% was achieved in mealworm. The production of Mr Xoch8.1 conidia in solid fermentation is a sustainable alternative through the use of inexpensive substrates and controlled conditions, such as a temperature of 28 °C, moisture of 60%, and absence of forced aeration. These conditions allow a high production of conidia with good germination, viability, and infectivity, demonstrating their potential as a biological control agent.

Palablas clave:

Metarhizium robertsii Xoch 8.1, Tenebrio molitor, biological control, conidia, infectivity.

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Introduction

The sustained increase in food demand, driven by population growth, has motivated the exploration of new strategies to optimize agricultural production. A key measure in this context is the reduction of crop losses, which are attributable to the action of phytopathogenic organisms, such as insects, nematodes, and fungi harmful to plants. Among the most widely adopted strategies, the use of agrochemicals has proven to be fundamental in mitigating the economic impact of these pests as it plays a decisive role in preserving and increasing agricultural yields.

Despite the benefits of certain agrochemicals, their toxicity, environmental persistence, and incorrect application has led to the need to reconsider pest control strategies (Guédez *et al*., 2008). In addition, pesticides are also potentially toxic to humans, and can cause cancer, damage to the brain, and changes in the fetus. The excessive use of pesticides in the country's agricultural areas is related to negative effects on terrestrial and coastal ecosystems (García-Hernández *et al*., 2018).

To reduce the above effects, biological control agents are formulated, which consist of living organisms whose purpose is to reduce the population of pest insects and pathogens that negatively impact crops. The use of entomopathogenic fungi, bacteria, viruses, and nematodes as biological control agents offers a promising alternative. These organisms can regulate insect, pests and pathogen populations effectively without the risks associated with conventional agrochemicals.

In addition, their ability to control pests in a specific manner reduces the negative impact on other non-target organisms and the environment in general (Sharma *et al*., 2023). Companies and research organizations are particularly interested in fungi due to their ability to play a role in controlling pests and diseases in crops, without causing damage to the environment or health (Nava-Pérez *et al*., 2012).

The optimization of culture conditions for the mass production of infective cells of organisms used in biological control is essential for their commercial viability. Understanding the factors that affect the growth and reproduction of these organisms, such as entomopathogenic fungi, is critical to maximizing the efficacy of bioinsecticide products (Jackson *et al*., 2010). Identifying and adjusting optimal growing conditions is crucial. This includes factors such as temperature, moisture, pH of culture medium, and nutrient composition. Variability in these conditions can significantly affect the rate of growth and reproduction (Xing *et al*., 2023).

The availability of nutrients, such as carbohydrates and proteins, is essential for microbial growth. Understanding how the organism uses and harnesses these nutrient sources can help design more efficient culture media (Jaronski, 2023). Assessing the growth rate of the organism at different temperatures and on various substrates provides valuable information about its versatility and adaptability. This allows us to select strains that perform well in a variety of conditions. Understanding the organism's metabolism is crucial.

The rate at which an organism reproduces and multiplies is a key factor (Parveen and Jeyarani, 2023). Fungi with faster growth rates may have competitive advantages in large-scale production and field application (Quesada-Moraga *et al*., 2023). This research aimed to evaluate the culture conditions (substrates, temperature, moisture, and aeration rate) in the production of *Metarhizium robertsii* conidia in solid cultures, as well as to analyze the quality of infective cells (germination, viability, and infectivity) at the laboratory level.

Materials and methods

Microorganisms, propagation, and conservation

The study was conducted with the Mr Xoch8.1 strain (*Metarhizium robertsii*) obtained from the fungal collection of the Laboratory of Enzymology and Molecular Biology of Filamentous Fungi of the Metropolitan Autonomous University (Iztapalapa, Mexico). The strain was propagated by streaking in oat agar medium (Tlecuitl-Beristain *et al*., 2010), the composition of which was as follows (g L^{-1}): 33.33 oats (one), 10 meat peptone (Bioxon, Mexico), and 15 bacteriological agar (Bioxon, Mexico); 100 x 15 mm Petri dishes were used with 20 ml of culture medium in each dish, and sterilization conditions were 121 °C and 15 psi for 20 min.

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The strain was incubated at 28 °C for 10 days; subsequently, it was preserved and the technique consisted of cutting fragments of sporulated agar of approximately 1 x 1 cm^2, which were placed in 15 ml tubes containing 5 ml of previously sterilized deionized water; finally, the tubes were stored at 4 °C (López-Lastra *et al*., 2022).

Conidiation profile

Glass column reactors with an internal diameter of 2 cm and a height of 20 cm were used as experimental units, and 10 g of initial dry substrate was used in each reactor. The substrate used was precooked rice with a moisture content of 1.96% before sterilization (Méndez-González *et al*., 2018), it was sterilized at 121 °C, 15 psi for 20 min; subsequently, the substrate was inoculated with a suspension of conidia at a concentration of 1 x 10^7 conidia ml^{-1}, the initial moisture was adjusted to 40% by adding sterile distilled water, and all experimental units were incubated in a water bath at 28 °C for 11 days (Angel-Cuapio *et al*., 2015).

Conidia were counted every 24 h from a column, which was considered as an independent experimental unit. The analyses were carried out in triplicate on different dates; the conidia were extracted using all the solid matter contained (10 g) in each column and were harvested by adding 60 ml of a Tween 80 solution (0.05%) (Amresco, Ohio, USA), and it was stirred for 10 min with a magnetic stirrer at 350 rpm.

After stirring the sample, it was filtered with a sterile gauze of 10 x 10 cm to separate the rice particles (Angel-Cuapio and Loera, 2016); from day 3 to day 11, the conidia were counted in a Neubauer chamber (Marienfield, Germany) under a microscope (Velab VE-M5LCD) and a 40X objective. Conidia production was reported as conidia per gram of initial dry substrate (conidia $gids^{-1}$).

Effect of substrate, temperature, moisture, and aeration rate on conidia production

Column reactors with rice were prepared to evaluate the effect of substrate, moisture, temperature, and aeration rate on conidia production; the conditions of sterilization, inoculation, incubation, extraction, and conidia counting were performed as previously described; all cultures were performed during eight days of culture (day of highest conidia production).

To evaluate the effect of the substrate, the following were used: precooked rice (verde valle), Peruvian beans (verde valle), white rice (verde valle), lentils (La Merced), popcorn (La Merced), millet (Los arbolitos, Sinaloa, Mexico), and wheat bran (Los arbolitos, Sinaloa, Mexico). Moisture contents of 20 to 70% were evaluated to determine the effect of the initial moisture of the substrate on the production of conidia.

To evaluate the effects of temperature during culture, incubation temperatures of 24 to 32 °C were evaluated. Finally, the effect of the aeration rate on the production of conidia was evaluated; for this, humid air flows of 0 to 50 ml air min^{-1} were assessed (Méndez-González *et al*., 2022).

Germination and viability

The percentage of germination was calculated using conidia obtained at eight days of culture. Petri dishes were prepared with Sabouraud dextrose agar medium, SDA (Bioxon, Mexico); conidia suspensions were standardized at a concentration of 1×10^6 conidia ml^{-1} and 50 µl of this suspension was inoculated in the Petri dishes and distributed with a glass rod; the dishes were incubated at 28 °C for 12 h. The conidia were counted considering the germinated and non-germinated ones and the observation was made under a microscope (VELAB VE-M5LCD) with a 40X objective.

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A germinated conidium was considered to be one that had a germ tube length of 2 times the diameter of the conidium (Ibrahim *et al*., 2002). The test was performed in triplicate. To determine the viability of the conidia, they were harvested at eight days of culture, conidia suspensions (1×10^4 conidia ml^{-1}) were standardized, and 30 µl (300 conidia) was added to Petri dishes with SDA medium; they were then incubated at 28 °C and colony-forming units (CFU) were counted at 48 h of culture (Alcantara-Vargas *et al*., 2020). The test was performed in triplicate.

Infectivity tests

For infectivity tests, larvae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) were used as model insects; the conidia produced in each column reactor were evaluated as follows: 10 *T. molitor* larvae were placed in each dish, eight Petri dishes were used for treatment (five dishes for infected larvae and three dishes as a negative control), infection was carried out by immersion of the larvae for 10 s in suspensions standardized at a concentration of 1×10^7 conidia ml^{-1}, which were prepared in tubes with a capacity of 50 ml.

For negative controls, larvae were immersed for 10 s in a sterile solution of Tween 80 (0.05%). The infectivity parameters were estimated by using the exponential decay equation (Rodríguez-Gómez *et al*., 2009):

$$Y=(100-S_f)e^{-k(t-t_0)}+S_f \text{for } t>t0$$

Where: Y is the percentage of survival (%) at time t, k is the specific death rate (d^{-1}), t_0 is the time when the first dead larva appeared (d) and S_f is the estimated asymptotic survival (%).

Statistical analysis

A one-way analysis of variance (Anova) was used with Tukey's test (95% confidence level); the average values of the experimental data were compared using the IBM SPSS Statistics 21 software (SPSS, Chicago, IL).

Results and discussion

Conidiation profile

The production of conidia as a function of time is greater at eight days after culture, with a value of 1.3×10^9 conidia $gids^{-1}$, and remains constant (8.91×10^8 conidia $gids^{-1}$) from days 9 to 11 of incubation (Figure 1). Therefore, the following experiments were performed at this incubation time.

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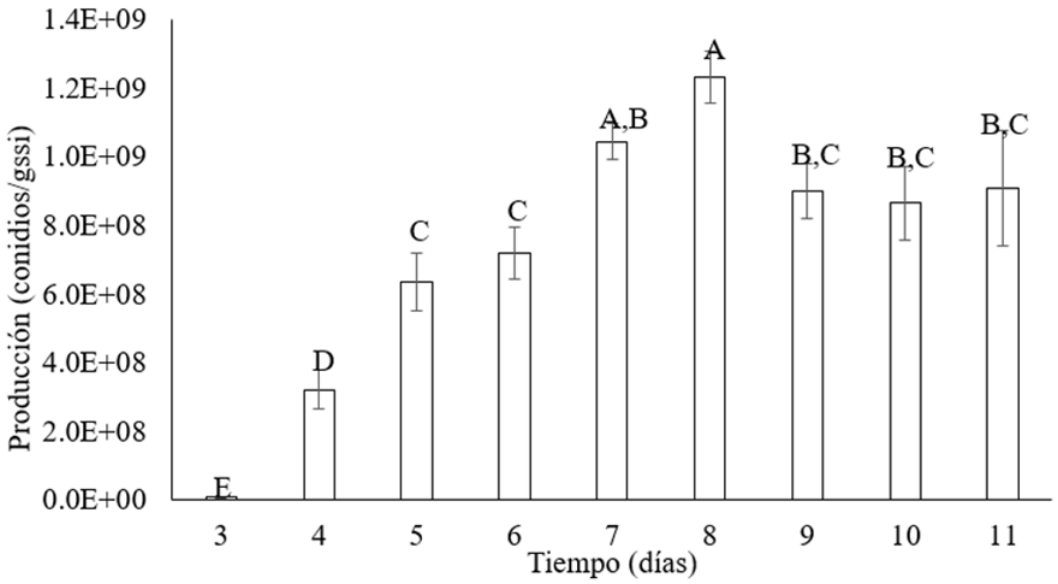
Figure 1. Conidiation profile of *Metarhizium robertsii* in solid cultures. Different letters differ significantly according to Tukey's test at the 95% confidence level.



Effect of substrate, temperature, moisture and aeration rate on conidia production

Nine solid substrates were evaluated for the production of *Metarhizium robertsii* conidia (Figure 2); the highest production value was reached when using white rice (1.05×10^9 conidia $gids^{-1}$), the value obtained was statistically significant ($p< 0.05$) compared to the results found in other evaluated substrates (millet, sorghum, popcorn, wheat, and lentils). Therefore, the following experiments were performed at eight days of incubation using white rice.

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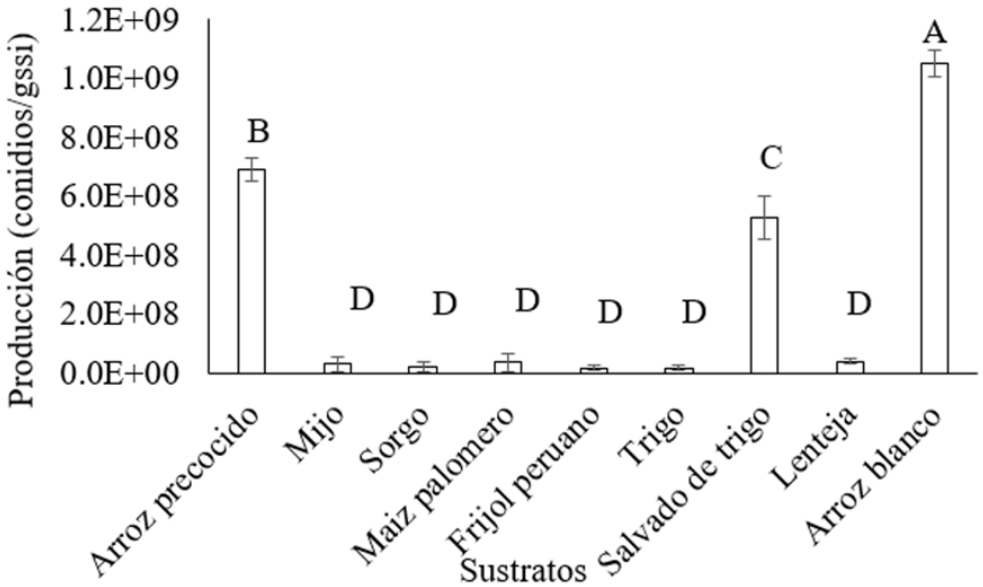
Figure 2. Production of *Metarhizium robertsii* conidia on different solid substrates. Different letters differ significantly according to Tukey test at the 95% confidence level.



Subsequently, the incubation temperature was analyzed and it was found that the highest production of conidia was reached at 28 °C, showing a significant difference ($p< 0.05$) between the analyzed temperatures, with a production of 1.04 x 10^9 conidia $gids^{-1}$; in contrast, no conidia production was observed at 32 °C (Figure 3). Therefore, the following experiments were performed at 8 days of incubation using white rice with an incubation temperature of 28 °C.

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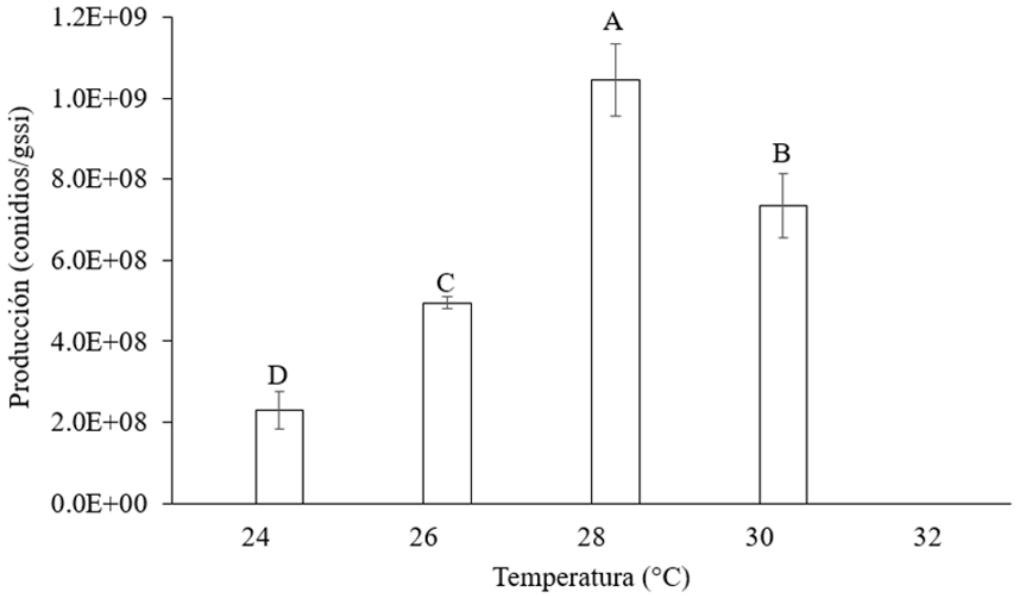
Figure 3. Production of *Metarhizium robertsii* conidia at different culture temperatures. Different letters differ significantly according to Tukey test at the 95% confidence level.



Because *M. robertsii* is an aerobic microorganism, the incorporation of moist air at different aeration rates was evaluated on conidia production (Figure 4). Increasing the rate of aeration decreases the production of conidia. The highest production was obtained with 0 ml min^{-1} (6 x 10^8 conidia $gids^{-1}$), being statistically different among the other air flows evaluated ($p< 0.05$). Therefore, the following experiments were performed at eight days of incubation using white rice with an incubation temperature of 28 °C and no airflow.

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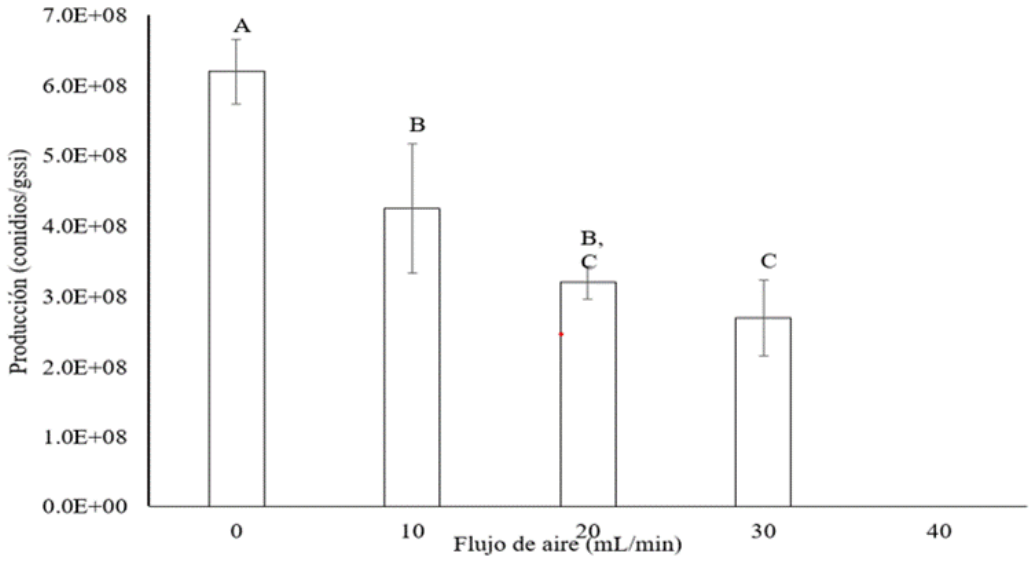
Figure 4. Production of *Metarhizium robertsii* conidia at different aeration rates. Different letters differ significantly according to Tukey's test at the 95% confidence level.



Different percentages of initial moisture were evaluated in the culture (Figure 5); it was found that when the moisture increases, the production of conidia increases; however, there is a moisture value in which the production of conidia is greater; in that sense, a production of 9.44 x 10^8 conidia $gids^{-1}$ was observed with a moisture of 60%, this result being statistically different (p# 0.05) among the other treatments.

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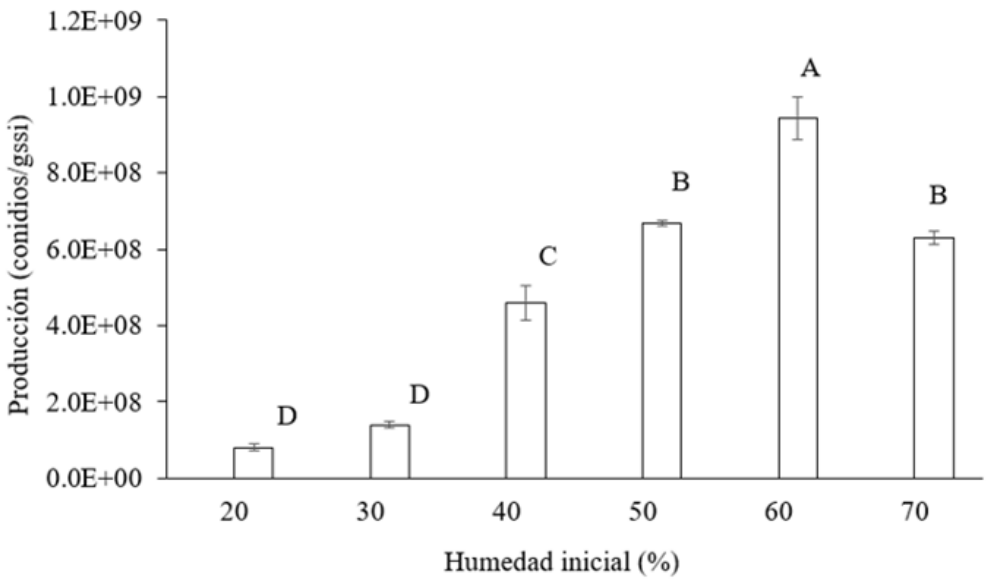
Figure 5. Production of *Metarhizium robertsii* conidia at different percentages of initial moisture. Different letters differ significantly according to Tukey's test at the 95% confidence level.



Germination and viability

Germination, viability (Figure 6) and infectivity were determined in order to measure the quality of the conidia obtained at eight days of incubation using white rice with an incubation temperature of 28 °C without forced aeration and an initial moisture of 60%. A value of 93% was found for germination, whereas a value of 57% was found for viability.

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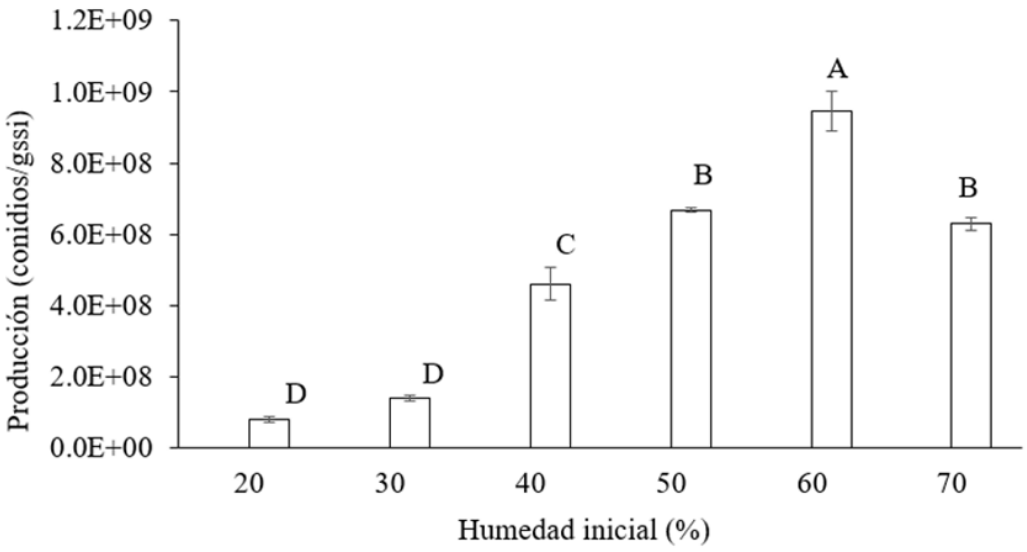
Figure 6. Viability and germination of conidia produced by the fungus *Metarhizium robertsii*



Infectivity tests

Regarding the infectivity parameters, the values obtained were a delay time (t_0) of 3.5 d, with a mortality rate (k) of 0.17 d^{-1}, a lethal time 50 (LT_{50}) of 15 d, and a final survival (S) of 40%.

Different conidia production values have been reported for the entomopathogenic fungus *Metarhizium*, such values indicate that the yields achieved depend on the culture conditions (pH, moisture, temperature, aeration, substrate, type of reactor, etc.) (Castillo-Castillo *et al*., 2022).

In this sense, one of the most economical substrates used in solid fermentation with a high percentage of starch (80%) is rice (Latifian *et al*., 2013). Angel-Cuapio and Loera (2016) report a production of *Metarhizium anisopliae* CP-OAX conidia of 5 x 10^8 conidia $gids^{-1}$ after six days of culture using a mixture of precooked rice with wood shavings as substrate.

For their part, García-Cruz *et al*. (2019) reported a conidia production of 8.9 x 10^8 conidia g^{-1} rice with the Ma-005 strain of *M. anisopliae* at 14 days of incubation. On the other hand, Méndez-González *et al*. (2018) analyzed the production of *M. anisopliae* conidia in bag bioreactors and obtained a production of 7 x 10^8 conidia g^{-1} rice at 11 days of culture. Likewise, da Cunha *et al*. (2019) studied the production of *M. anisopliae* conidia in tray bioreactors and observed a production of 2.8 x 10^9 conidia g^{-1} rice during 10 d of culture.

On the other hand, Prakash *et al*. (2008) conducted research to optimize the production of *M. anisopliae* conidia in polypropylene bags and found a yield of 6.2 x 10^{10} conidia g^{-1} rice at 15 days of incubation. Temperature is the key factor limiting the effective use of *Metarhizium*; Vishwanath *et al*. (2021) reported that *Metarhizium anisopliae* presents a better conidiation at a temperature of 30 °C at siete days of incubation, which is consistent with what was obtained in the present research; likewise, it has been found that the genus *Metarhizium* is considered a mesophilic microorganism that can grow in a temperature range between 15 and 35 °C; however, optimal growth is between 25 and 30 °C (Zimmermann, 2007).

Regarding the air supply, it was found that high aeration rates negatively affected the conidiation of *M. robertsii*, which is consistent with what was reported by Arzumanov *et al*. (2005) with the strain IMI330189 of *Metarhizium anisopliae*, they found the highest production of conidia without airflow and they mention that forced air is not crucial for conidiation.

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On the other hand, entomopathogenic fungi are aerobic microorganisms that require an air supply for growth and conidiation (Muñiz-Paredes and Loera, 2006); Méndez-González *et al*. (2022) mention that the supply of forced convection aeration (0.33 L kg^{-1} min^{-1}) in column bioreactors presented high levels of production and productivity of *M. robertsii* conidia without affecting the quality of the cells obtained and they suggest that aeration between 0.1 and 0.66 L kg^{-1} min^{-1} is sufficient to effectively remove the CO_2 produced by the microorganism since the accumulation of CO_2 in the gaseous atmosphere of bioreactors affects the conidiation.

The germination and viability of conidia (asexual spores) of entomopathogenic fungi are crucial aspects for the success of these organisms as insect biological control agents, being an environmentally friendly alternative to chemical pesticides (Shahid *et al*., 2012). Conidia germination is influenced by environmental factors, such as temperature, moisture and light, but each species of entomopathogenic fungus may have specific requirements for efficient germination; nevertheless, it is essential for understanding and optimizing applications in the field (Benítez *et al*., 2004).

The present study found a germination of 93% and a viability of 40%, which are values consistent with those reported by other authors, as mentioned by Ruiz-Sánchez *et al*. (2011), who, with the *M. anisopliae* strain, found a germination of 100% at six hours of incubation with a suspension of 1 x 10^7 conidia ml^{-1}, considering as germinated conidium those that have the length of the germ tube equal to the size of the conidium; on the other hand, Alcantara-Vargas *et al*. (2020) found a 50% viability for conidia of the *M. anisopliae* strain produced in rice.

The germination and viability parameters of entomopathogenic fungi comply with the standards established for the formulation of mycoinsecticides (Jenkins and Grzywacz, 2000); these standards are specific parameters that indicate the suitability of the strains for the production of biopesticides. The conidia of entomopathogenic fungi are usually the infective form for insects, hence the importance of producing them in large quantities and evaluating their quality; however, there are different factors that influence effectiveness, such as the mechanisms of infection that consider cuticular penetration and the production of enzymes and toxins (Jiubari *et al*., 2023)

Also important is the specificity of the host, which involves how conidia attach, germinate, and penetrate the insect, and the mechanisms that the insect may trigger in response to infection (Wang *et al*., 2023). Another feature to mention has to do with environmental factors since infectivity can depend on environmental factors, such as temperature, moisture and nutrient availability (Xing *et al*., 2023).

Conclusions

The best culture conditions for the production of *Metarhizium robertsii* conidia in solid fermentation were identified. The nature of the substrate is very important for the production of conidia. White rice led to the highest production. Of the variables evaluated, incubation temperature and aeration had the greatest impact on conidia production.

Under the production conditions, the conidia of Mr Xoch8.1 presented a high quality, with germination, viability, and infectivity rates sufficient to cause 60% mortality in mealworm larvae, which demonstrated their biological efficacy as a control agent. The suggestion to evaluate the possibility of simultaneous application with other fungi is interesting since synergies between different fungal strains could improve the effectiveness of biological control and expand the range of target species. Furthermore, if this can be achieved without significant additional costs, it could be a viable strategy.

The low cost of production is a key factor that can make the application of *Metarhizium robertsii* economically viable. This is essential to consider its large-scale application in agriculture. The proposal to continue research in this field is crucial. Expanding knowledge about the mass production of *Metarhizium robertsii* could lead to improvements in the efficacy, stability, and applicability of the fungus in different agricultural contexts.

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Bibliography

1 Alcantara-Vargas, E.; Espitia-López, J.; Garza-López, P. M. and Angel-Cuapio, A. 2020. Conidia production and quality of entomopathogenic strains of the genus *Metarhizium anisopliae*, isolated in agricultural zones of the State of Mexico. México. Revista Mexicana de Biodiversidad. (91):1-11. 10.22201/ib.20078706e.2020.91.2912.

2 Angel-Cuapio, A. and Loera, O. 2016. Uso de residuos agroindustriales como texturizantes para la producción de hongos entomopatógenos en cultivo en estado sólido. México. Mexican Journal of Biotechnology. 1(1):21-33.

3 Angel-Cuapio, A.; Figueroa-Montero, A.; Favela-Torres, E.; Viniegra-González, G.; Perraud-Gaime, I. and Loera, O. 2015. Critical values of porosity in rice cultures of *Isaria fumosorosea* by adding water hyacinth: effect on conidial yields and quality. México. Applied Biochemistry and Biotechnology. (177):446-457. 10.1007/s12010-015-1754-4.

4 Arzumanov, T.; Jenkins, N. and Roussos, S. 2005. Effect of aeration and substrate moisture content on sporulation of *Metarhizium anisopliae* var. *acridum.* Reino Unido. Process Biochem. 40(3-4):1037-1042. 10.1016/j.procbio.2004.03.013.

5 Benítez, T.; Rincón, A. M.; Limón, M. C. and Codon, A. C. 2004. Biocontrol mechanisms of *Trichoderma* strains. España. International Microbiology. 7(4):249-260.

6 Castillo-Castillo, H.; Rojas-Gutiérrez, L.; Espitia-López, J.; Garza-López, P.; Martínez-de Jesús, G.; Neria-González, I. and Angel-Cuapio, A. 2022. Texturizers increase the conidia production of *Metarhizium anisopliae.* México. Mexican Journal of Technology and Engineering. 2(1):22-29. https://doi.org/10.61767/mjte.001.2.2229.

7 Da Cunha, L. P.; Casciatori, F. P.; Cenço, L. I. and Thoméo, J. C. 2019. Production of conidia of the entomopathogenic fungus *Metarhizium anisopliae* ICB 425 in a tray bioreactor. Brasil. Bioprocess and Biosystems Engineering. 11(42):1757-1768. https://doi.org/10.1007/s00449-019-02172-z.

8 García-Cruz, I.; del Pozo-Núñez, E. M. and Hernández-Pérez, Y. 2019. Producción y conservación de conidios del aislado Ma-005 de *Metarhizium anisopliae* (Metsch.) Sorokin. Cuba. Centro Agrícola. 1(46):5-12.

9 García-Hernández, J.; Leyva-Morales, J. B.; Martínez-Rodríguez, I. E.; Hernández-Ochoa, M. I.; Aldana-Madrid, M. L.; Rojas-García, A. E.; Betancourt-Lozano, M.; Perez-Herrera, N. E. and Perera-Rios, J. H. 2018. Estado actual de la investigación sobre plaguicidas en México. México. Revista Internacional de Contaminación Ambiental. 34(1):29-60. 10.20937/RICA.2018.34.esp01.03.

10 Guédez, C.; Castillo, C.; Cañizales, L. and Olivar, R. 2008. Biological control is a tool for sustaining and sustainable development. Venezuela. Academia. 7 (13):50-74.

11 Ibrahim, L.; Butt, T. M. and Jenkinson, P. 2002. Effect of artificial culture media on germination, growth, virulence and surface properties of the entomopathogenic hyphomycete *Metarhizium anisopliae.* Reino Unido. Mycological Research. 106(6):705-715. 10.1017/S0953756202006044.

DOI: https://doi.org/10.29312/remexca.v16i3.3596

12 Jackson, M. A.; Dunlap, C. A. and Jaronski, S. T. 2010. Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. Biocontrol. 55(1):129-145. 10.1007/s10526-009-9240-y.

13 Jaronski, S. T. 2023. Mass production of entomopathogenic fungi-state of the art. *In*: mass production of beneficial organisms: invertebrates and Entomopathogens. Morales, J. A., Ed. 2nd. Academic Press. USA. 317-357 pp. 10.1016/B978-0-12-822106-8.00017-8.

14 Jenkins, N. E. and Grzywacz, D. 2000. Quality control of fungal and viral biocontrol agents-assurance of product performance. Brazil. Biocontrol Science and Technology. 6(10):753-777. https://doi.org/10.1080/09583150020011717.

15 Latifian, M.; Rad, B.; Amani, M. and Rahkhodaei, E. 2013. Mass production of entomopathogenic fungi *Beauveria bassiana* (Balsamo) by using agricultural products based on liquid-solid diphasic method for date palm pest control. Iran. International Journal of Agriculture and Crop Sciences. 5 (19):2337-2341. https://doi.org/10.5897/AJB07.778.

16 López-Lastra, C. C.; Hajek, A. E. and Humber, R. A. 2002. Comparing methods of preservation for cultures of entomopathogenic fungi. Canada. Canadian Journal of Botany. 80 (10):1126-1130. https://doi.org/10.1139/b02-090.

17 Méndez-González, F.; Figueroa-Montero, A.; Loera, O.; Saucedo-Castañeda, G. and Favela-Torres, E. 2022. Addition of spherical-style packing improves the production of conidia by *Metarhizium robertsii* in packed column bioreactors. Reino Unido. Journal of Chemical Technology & Biotechnology. 97 (6):1517-1525. 10.1002/jctb.6993.

18 Méndez-González, F.; Loera, O. and Favela-Torres, E. 2018. Conidia production of *Metarhizium anisopliae* in bags and packed column bioreactors. Brazil. Current Biotechnology. 7 (1):65-69. 10.2174/2211550105666160926123350.

19 Muñiz, F. R. and Loera, O. 2006. The importance of strong inoculum in fungal cultures. México. Mexican Journal of Biotechnology. 1(1):120-134.

20 Nava-Pérez, E.; García-Gutiérrez, C.; Camacho-Báez, J. R. and Vázquez-Montoya, E. L. 2012. Bioplaguicidas: una opción para el control biológico de plagas. Ra Ximhai. 8(3):17-29. 10.35197/rx.08.03.e2.2012.03.en.

21 Parveen, S. S. and Jeyarani, S. 2023. Laboratory evaluation of temperature effects on germination, radial growth and sporulation of entomopathogenic fungi and on their pathogenicity to red spider mite, *Tetranychus urticae* Koch. India. Indian Journal of Agricultural Research. 57 (3):376-382. 10.1002/ps.622.

22 Prakash, G. B.; Padmaja, V. and Kiran, R. S. 2008. Statistical, optimization of process variables for the large-scale production of *Metarhizium anisopliae* conidiospores in solid-state fermentation. India. Bioresource Technology. 99(6):1530-1537. 10.1016/ j.biortech.2007.04.031.

23 Quesada-Moraga, E.; González-Mas, N.; Yousef-Yousef, M.; Garrido-Jurado, I. and Fernández-Bravo, M. 2023. Key role of environmental competence in successful use of entomopathogenic fungi in microbial pest control. España. Journal of Pest Science. 97(1):1-15. 10.1007/s10340-023-01622-8.

24 Rodríguez-Gómez, D.; Loera, O.; Saucedo-Castañeda, G. and Viniegra-González, G. 2009. Substrate influence on physiology and virulence of *Beauveria bassiana* acting on larvae and adults of Tenebrio molitor. México. World Journal of Microbiology and Biotechnology. 25:513-518. 10.1007/s11274-008-9917-x.

25 Ruiz-Sánchez, E.; Chan-Cupul, W.; Pérez Gutiérrez, A.; Cristóbal-Alejo, J.; Uch-Vazquez, B.; Tun-Suárez, J. M. and Munguía-Rosales, R. 2011. Crecimiento, esporulación y germinación *in vitro* de cinco cepas de *Metarhizium* y su virulencia en huevos y ninfas de *Bemisia tabaci*. México. Revista Mexicana de Micología. 33:9-15.

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26 Shahid, A. A.; Rao, A. Q.; Bakhsh, A. and Husnain, T. 2012. Entomopathogenic fungi as biological controllers: new insights into their virulence and pathogenicity. Pakistán. Archives of Biological Sciences. 1(64):21-42. https://doi.org/10.2298/ABS1201021S.

27 Sharma, A.; Sharma, S. and Yadav, P. K. 2023. Entomopathogenic fungi and their relevance in sustainable agriculture: A review. Nepal. Cogent Food & Agriculture. 9(1):2180857. https://doi.org/10.1080/23311932.2023.2180857.

28 Tlecuitl-Beristain, S.; Viniegra-González, G.; Díaz-Godínez, G. and Loera, O. 2010. Medium selection and effect of higher oxygen concentration pulses on *Metarhizium anisopliae* var. *lepidiotum* conidial production and quality. México. Mycopathologia. 169(5):387-394. 10.1007/s11046-009-9268-7.

29 Vishwanath, P. P.; Ahire, R. S.; Patil, S. R. and Pawar, S. B. 2021. Isolation, identification and evaluation of media for *Metarhizium anisopliae* growth and sporulation. India. Educational Research (IJMCER). 3 (4):250-258.

30 Wang, J. B.; Lu, H. L.; Sheng, H. and St. Leger, R. J. 2023. A *Drosophila melanogaster* model shows that fast growing *Metarhizium* species are the deadliest despite eliciting a strong immune response. Estados Unidos. Virulence. 14(1):2275493. 10.1080/21505594.2023.2275493.

31 Xing, P.; Diao, H.; Wang, D.; Zhou, W.; Tian, J. and Ma, R. 2023. Identification, pathogenicity, and culture conditions of a new isolate of*Cordyceps javanica*(Hypocreales: Cordycipitaceae) from soil. Estados Unidos. Journal of Economic Entomology. 116(1):98-107. 10.1093/jee/toac199.

32 Zimmermann, G. 2007. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Canadá. Biocontrol Science and Technology. 17(9):879-920. 10.1002/ps.2780370410.

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Solid fermentation of *Metarhizium robertsii*: substrate and culture conditions on conidia production and the biological efficacy

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