Article **Journal of Biomedical Engineering and Biotechnology**
December, 2023 Vol.7 No.18 13-21

Comparison of the effect of oxidant pulses on conidia under culture and free conidia

Comparación del efecto de pulsos oxidantes sobre conidios bajo cultivo y conidios libres

CASTILLO-MINJAREZ, José Miguel Angel†*, MARTÍNEZ-VALDEZ, Francisco Javier, CHÁVEZ-ESCALANTE, Luis Geovani and GÓMEZ-SÁNCHEZ, Ángel David

División de Electromecánica Industrial, Universidad Tecnológica de Tecámac. CP 55740. México.

ID 1st Author: *José Miguel Angel, Castillo-Minjarez* / **ORC ID**: 0000-0001-8647-5303

ID 1st Co-author: *Francisco Javier, Martínez-Valdez* / **ORC ID**: 0000-0002-1640-5577

ID 2nd Co-author: *Luis Geovani, Chávez-Escalante* / **ORC ID**: 0009-0004-3691-3589

ID 3rd Co-author: *Ángel David, Gómez-Sánchez* / **ORC ID**: 0000-0003-2919-3806

DOI: 10.35429/JBEB.2023.18.7.13.21 Received November 10, 2023; Accepted December 30, 2023

Abstract

The objectives of sustainable development demand ecosystem-friendly agricultural practices, so the use of entomopathogenic fungi as a technology for pest control is very convenient. Oxidant pulses are an alternative method for improving the quality of conidia that could be implemented in the production chain. However, it has not been determined at what time their implementation could be more convenient, either during the cultivation stages or when the conidia have already been separated from the culture medium. Therefore, the objective of this study was to compare for the first time the effect of oxidant pulses on the quality of conidia of entomopathogenic fungi, making a simultaneous evaluation in both conditions (conidia in culture medium vs. free conidia). Using three genera of fungi of common commercial use, under the study conditions it is concluded that it is better to apply the treatment on free conidia of *Beauveria bassiana* and *Metarhizium robertsii*. On the contrary, with *Cordyceps fumosorosea*, it is better to apply the treatment on the conidia that remain in the culture medium.

Biopesticides, Sustainable Agriculture, Conidia

Resumen

Los objetivos del desarrollo sostenible demandan prácticas agrícolas amigables con los ecosistemas, por lo que el uso de hongos entomopatógenos como tecnología para el control de plagas resulta muy conveniente. Los pulsos oxidantes son una alternativa como método de mejora de la calidad de los conidios que podría ser implementado en la cadena de producción. Sin embargo, no se ha determinado en qué momento podría ser más conveniente su implementación, ya sea durante las etapas de cultivo o cuando los conidios ya han sido separados del medio de cultivo. Por lo tanto, el objetivo de este estudio fue comparar por primera vez el efecto de pulsos oxidantes sobre la calidad de conidios de hongos entomopatógenos, haciendo una evaluación simultánea en ambas condiciones (conidios en medio de cultivo vs conidios libres). Empleando tres géneros de hongos de uso comercial común, bajo las condiciones de estudio se concluye que, es mejor aplicar el tratamiento sobre los conidios libres de Beauveria bassiana y Metarhizium robertsii. Por el contrario, con Cordyceps fumosorosea, es mejor aplicar el tratamiento sobre los conidios que se mantienen en el medio de cultivo.

Bioplaguicidas, Agricultura Sostenible, Conidios

Citation: CASTILLO-MINJAREZ, José Miguel Angel, MARTÍNEZ-VALDEZ, Francisco Javier, CHÁVEZ-ESCALANTE, Luis Geovani and GÓMEZ-SÁNCHEZ, Ángel David. Comparison of the effect of oxidant pulses on conidia under culture and free conidia. Journal of Biomedical Engineering and Biotechnology. 2023. 7-18: 13-21

* Author Correspondence (e-mail: jcastillom@uttecamac.edu.mx)
† Researcher contributing as first author.

Introduction

Achieving the sustainability vision outlined in the 2030 Agenda demands a transformative shift in how we apply new technologies, particularly in agriculture. As United Nations (UN) Secretary-General António Guterres stated, "We must transform the way we produce, consume, and waste food." Sustainable Development Goal 2 (SDG2) demands that we end hunger and achieve food security by 2030. This includes not only improved nutrition and eradicated hunger, but also fostering sustainable agricultural practices that protect ecosystems (United Nations, 2018). One promising approach to achieving these interconnected goals lies in utilizing entomopathogenic fungal conidia as biopesticides for pest control. This offers a safer and more environmentally friendly alternative to conventional chemical pesticides (Méndez-González *et al*., 2022; Miranda-Hernández *et al*., 2016).

The success of biopesticides in the field, hinges on the diverse arsenal of traits possessed by the fungal conidia. These adaptations equip them to overcome numerous environmental challenges, including insect defense mechanisms, harsh temperatures, salinity fluctuations, humidity extremes, and ultraviolet radiation. Essentially, a conidia's ability to navigate these hurdles determines its ultimate success as a biocontrol agent (Castillo-Minjarez *et al*., 2019; Miranda-Hernández *et al*., 2016).

Desirable quality conidial traits for biocontrol, such as high virulence, thermotolerance, and resistance to osmotic stress and UV-B radiation, can be influenced by the fungus's growth conditions (Rangel *et al*., 2015). Notably, this ability to adapt is particularly relevant in the context of oxidative stress, which can be induced by sublethal treatment with gaseous pulses rich in oxygen (oxidant pulses). This adaptation can offer significant benefits for conidial production. For instance, Tlecuitl-Beristain *et al*. (2010) found that exposing *Metarhizium anisopliae* to oxidant pulses with 26 % oxygen during growth resulted in a 100 % increase in conidial production without affecting germination, hydrophobicity, or infectivity against *Tenebrio molitor*. Such enhanced conidial traits hold promise for improving the efficacy and cost-effectiveness of biopesticides, leading to a more sustainable approach to pest control.

The impact of oxidant pulses on conidia varies depending on fungal species and treatment´s parameters. Studies reveal both positive and strain-specific outcomes. García-Ortiz *et al*. (2015) observed a remarkable increase in conidia production and enhanced thermotolerance in *Metarhizium anisopliae* exposed to 30 % oxygen pulses, without compromising viability, hydrophobicity, or infectivity. Conversely, Miranda-Hernández *et al*. (2014) reported contrasting responses in two *Cordyceps fumosorosea* (also known as *Isaria fumosorosea*), subjected pulses with 26 % oxygen: one strain exhibited a fivefold increase in conidia production, while the other showed a 50 % decrease. However, both strains displayed an improved infectivity against *Galleria mellonella* larvae, germination rate, and stress resistance thermal, and osmotic.

These divergent responses highlight the complex interplay between fungal biology and oxidative stress. Notably, the potential benefits of oxidant pulses extend beyond the growth phase. Notably, Castillo-Minjarez *et al.* (2019) demonstrated the benefits of oxidant pulses even for harvested conidia (free conidia), treating them with 26 % oxygen significantly increased osmotic stress resistance in *Metarhizium robertsii*, *Beauveria bassiana*, and one *Cordyceps javanica* strain. Additionally, germination and thermotolerance improved in *M. robertsii* and *B. bassiana.*

Driven by the escalating demand for high-quality, readily available conidia in mass production for biocontrol, oxidant pulses emerge as a promising tool for enhancing their efficacy. However, a crucial question lingers: The optimum time to apply the oxidant pulses for best results. The integration of oxidant pulses during the stages of fungal culture, while within the shelter of the culture medium, or application to the harvested conidia, already free of their nutrient source, are two options to be evaluated. Each approach presents distinct feasibility challenges and cost considerations. Cultivating under oxidative stress could demand stringent sterility measures, potentially inflating production expenses. Conversely, treating free conidia might be less costly but could potentially compromise conidial stability.

Navigating the optimal timing for oxidant pulses application requires discerning which stage yields the most potent effect.

ISSN 2523-6857

This study embarked on a mission to compare the impact of oxidant pulses on both cultivated and free conidia of three prominent entomopathogenic fungi (*Metarhizium*, *Beauveria*, and *Cordyceps*). The focus was on assessing if observed positive effects, such as enhanced thermotolerance and stress resistance, remained comparable across both application stages. This knowledge will unlock ways for future cost-benefit analyses and pave the way for the optimal integration of oxidant pulses into large-scale conidia production, ultimately enhancing the efficacy and affordability of sustainable pest control strategies.

Methodology

1. Biological material

The microorganisms used were *Cordyceps fumosorosea* ARSEF3302 obtained from the Agricultural Research Service Culture Collection of Entomopathogenic Fungi (ARSEF), Ithaca, New York; *Metarhizium robertsii* Xoch8.1 and *Beauveria bassiana* Tac1.1, obtained from the Centro Nacional de Referencia de Control Biológico, Colima, Mexico. The strains were deposited in the ENCBIPN Culture Collection WDCM449 with identification numbers ENCB-MG-79 (*C. fumosorosea*), ENCB-MG-81 (*M. robertsii*) and ENCB-MG-82 (*B. bassiana*).

2. Initial propagation

Fungi of each species studied were grown at 27 ± 2 °C in 250 mL Erlenmeyer flasks (Pyrex) containing the surface medium here called APA, composed of 33.3 g/L oat flour (Sol Campestre®, Productos del Campo, Mexico), 15 g/L bacteriological agar (Bioxon, Mexico) and 10 g/L meat peptone (Bioxon, Mexico) (Tlecuitl-Beristain *et al*., 2010).

3. Quantification and extraction of conidia on culture medium

After 8 to 9 days of incubation, the conidia in culture medium were extracted with 0.05 % Tween 80 (Hycel). The conidia were counted in a Neubauer chamber (Marienfeld, Lauda-Königshofen, Germany) and optical microscope (Boeco, Germany). From the conidial extracts with Tween 80, conidial suspensions of 1 x 10^8 con/mL were prepared, from which inocula were taken to perform the different experiments.

Under sterile conditions, 100 µl of the 1 x 10^8 con/ml suspensions were taken and inoculated into 120 mL serological bottles (Distbrand, Mexico) containing APA medium (Miranda-Hernández *et al*., 2014).

4. Conidiation status

It was proposed the introduction of a new parameter called Conidiation Index (*Ic*), which easily indicates how the production of conidia in the culture medium varies over time, with respect to an initial inoculum.

The parameter is calculated with the following equation:

$$Ic = \frac{C}{Ci} \quad (1)$$

Where:
C is the observed production of conidia in each time.
Ci is the concentration of the initial inoculum.
The units of both terms are conidia per square centimeter (con/cm^2).

The study employed 100 µL of 1 x 10^8 con/mL suspensions as inoculum in serological bottles containing APA medium (section 3). This corresponds to an initial concentration of 5.76 x 10^5 conidia per square centimeter of agar surface (con/cm^2), equivalent to an *Ic* = 1. Applying equation (1), a measured concentration of 1.80 x 10^6 con/cm^2 at a specific culture time would result in:

$$Ic = \frac{C}{Ci} = \frac{1.80 \text{ x } 10^6}{5.76 \text{ x } 10^5} = 3.12$$

This would indicate that the production of conidia increased 3.12 times with respect to the initial amount of conidia in the culture.

The effect of the oxidant pulses on conidia production was determined by comparing the *Ic* obtained under the different atmospheric conditions studied.

5. Oxidant pulses

An oxidant pulse consisted of inserting a needle into the rubber stopper that functioned as a gas outlet and a second needle was placed, which in turn was connected to the gas tank with the oxygen mixture corresponding to 26 %; once both needles were placed.

ISSN 2523-6857

The gas was allowed to pass for 1 min at a flow rate of 20 cm^3/s; subsequently, the needles were removed and the bottles were kept at room temperature. During the time each experiment lasted, the pulse was reapplied every 24 h (Castillo-Minjarez *et al*., 2019).

6. Assessing the effects of oxidant pulses on the quality of free conidia and conidia in culture medium

This experiment was carried out to observe the effect of oxidant pulses on the quality of conidia under culture and to compare with the effect on free conidia. The same methodologies were applied to all the fungi studied.

6.1 Harvesting conidia in culture medium and free conidia

A batch of serological bottles containing 10 mL of APA medium was made and inoculated with 100 µl of a 1 x 10^8 con/mL suspension and incubated at 27 $\pm$ 2 °C. Once cultures were at an advanced stage of conidiation (*Ic* close to 100), bottles were taken to exchange the cotton plug for the rubber stopper and start the pulse. Simultaneously, bottles were taken to scrape the conidia gently with a sterile spatula and a standardized amount of these was placed in sterile serological bottles, without culture medium, some bottles were kept with cotton plugs (control units) and others with rubber stopper to apply the oxidant pulses.

In all cases, the evaluation of the quality characteristics was made 24 h after starting the oxidant pulse and 96 h later (in the latter case, the pulses were applied every 24 h).

6.2 Viability

Under sterile conditions, 1 x 10^4 con/mL suspensions were prepared, 30 µL (300 conidia approximately) were taken and inoculated in Petri dishes with ADS (Bioxon, Mexico), incubated at 27 $\pm$ 2 °C for 60 h and the colony forming units (CFU) present were counted, determining the percentage of viability (Miranda-Hernández *et al*., 2014).

6.3 Heat resistance

The same suspensions (1 x 10^4 con/mL) used for viability were placed at 40 °C for 1 h in a thermomixer (Eppendorf, Germany).

After the time elapsed, the viability of conidia was determined as previously explained (Castillo-Minjarez *et al*., 2019).

6.4 Osmotic stress resistance

The viability of conidia from different atmospheric conditions and from different species was evaluated, as previously described, in this case the 30 µL of 1 x 10^4 con/mL suspensions were inoculated in Petri dishes with ADS (Bioxon, Mexico) and NaCl (Meyer, Mexico) 0.5 M (Castillo-Minjarez *et al*., 2019).

7 Statistical analyses

Each experiment was made by triplicate. Student's *t* test (α= 0.05 %) from the "Data Analysis" tool of Microsoft Excel (2010) was used.

Results

1. Effect of treatment on conidiation

Figures 1, 2 and 3 show the conidiation indexes (*Ic*) in the control cultures (normal atmosphere of 21 % oxygen) and with the oxidant pulses (26 % oxygen). It can be observed that in all cases a high conidiation state (close to or greater than 100) was present. In addition, a positive effect was observed on the treatment on production of conidia of the *Cordyceps fumosorosea* ARSEF3302 and *Metarhizium robertsii* Xoch8.1.

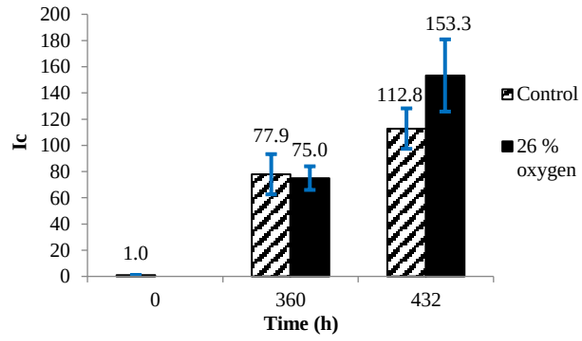


Figure 1 Effect of the oxidizing pulse on the production of conidia of the entomopathogenic fungus *Beauveria bassiana* Tac1.1
Own Source

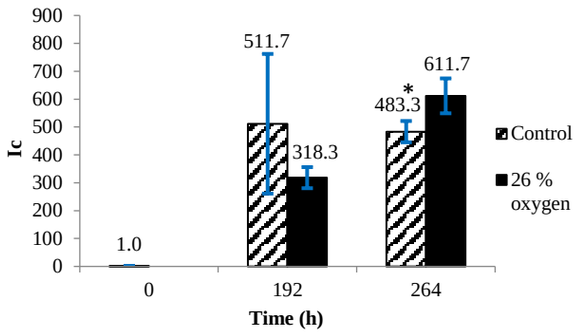


Figure 2 Effect of oxidative pulse on conidial production of the entomopathogenic fungus *Cordyceps fumosorosea* ARSEF3302, *statistically significant difference
Own Source

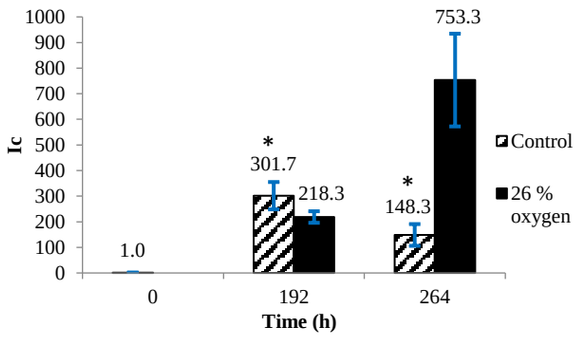


Figure 3 Effect of oxidative pulse on conidial production of the entomopathogenic fungus *Metarhizium robertsii* Xoch8.1, *statistically significant difference
Own Source

2. Evaluation of the effect of oxidative pulse on the quality of free conidia and conidia in culture medium

The following section presents the effects of the treatment on the evaluated quality characteristics, both in the cultured conidia and the free conidia.

2.1 Viability

In the case of *Beauveria bassiana* Tac1.1 (Figure 4), it is observed that the viability naturally decreased in conidia that were separated from the culture medium (free conidia) compared to those that remained in it. Regarding the effect of the treatment, it was observed that oxidant pulse improved viability in conidia that remained in the culture medium, and despite the drop in viability of free conidia, there was also an improvement in this characteristic when the first pulse was applied (24 h).

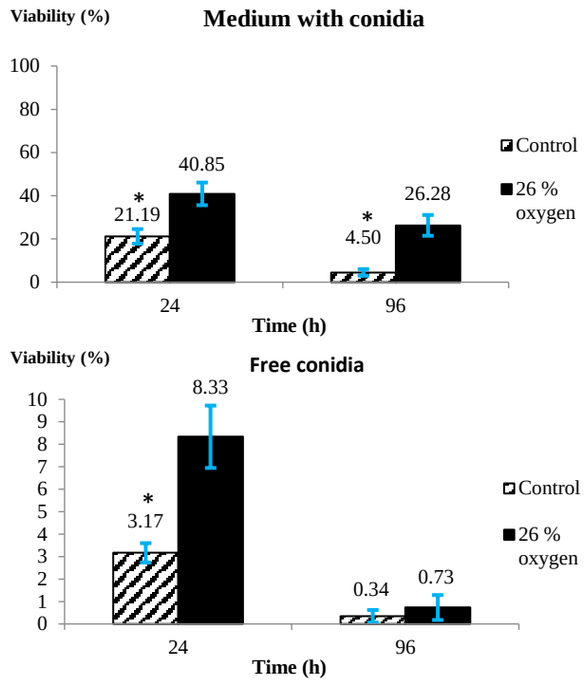


Figure 4 Effect of treatment on viability of *Beauveria bassiana* Tac1.1, *statistically significant difference
Own Source

In the case of *Cordyceps fumosorosea* ARSEF3302 (Figure 5), viability was very similar between free conidia and those maintained in the culture medium. However, this fungus presented a very low viability. Regarding the treatment effect, it was observed that the oxidant pulses improved viability in the conidia that remained in the culture medium; nevertheless, there was the opposite effect in the free conidia.

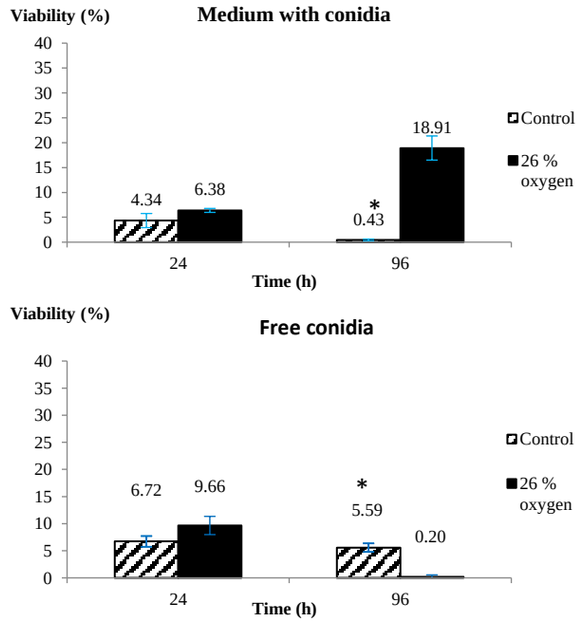


Figure 5 Effect of treatment on viability of *Cordyceps fumosorosea* ARSEF3302, *statistically significant difference
Own Source

ISSN 2523-6857

In the case of *Metarhizium robertsii* Xoch8.1 (Figure 6), it was observed that viability was very similar between free conidia and those kept in culture medium. Regarding the treatment effect, it was observed that the first oxidant pulse (24 h) improved viability in the conidia that remained in the culture medium. In contrast, free conidia showed no change.

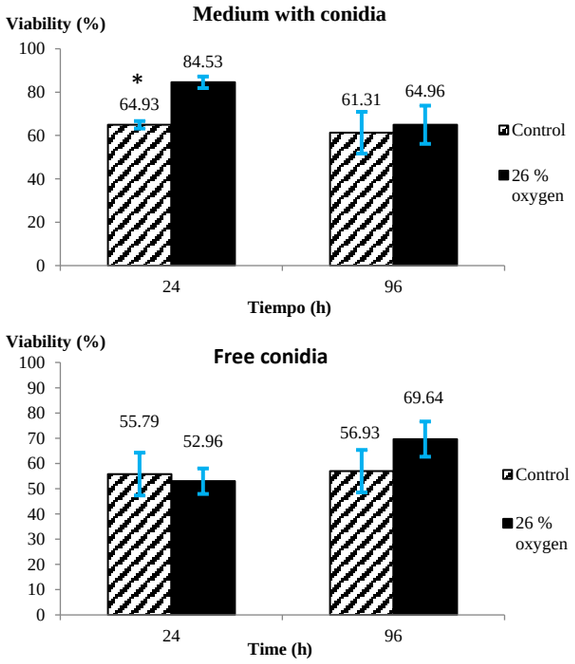
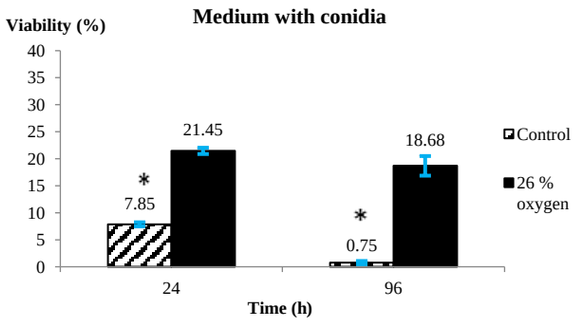


Figure 6 Effect of treatment on viability of *Metarhizium robertsii* Xoch8.1, *statistically significant difference
Own Source

2.2 Heat resistance

In the case of *Beauveria bassiana* Tac1.1 (Figure 7), it was observed that the oxidant pulses improved the high temperature resistance in the conidia that remained in the culture medium and despite the drop in viability of the free conidia; there was also an improvement in the high temperature resistance when the first pulse (24 h) was applied.



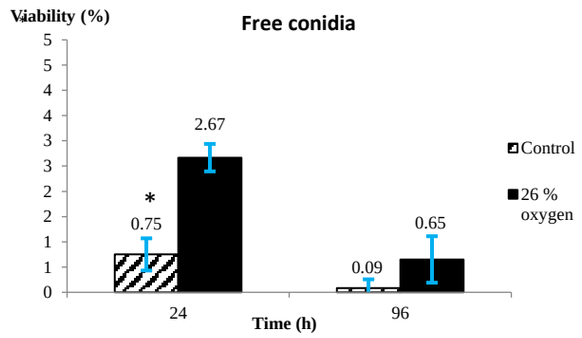


Figure 7 Effect of treatment on high temperature resistance of *Beauveria bassiana* Tac1.1, *statistically significant difference
Own Source

In the case of *Cordyceps fumosorosea* ARSEF3302 (Figure 8), it was observed that the oxidant pulses improved the resistance to high temperature in the conidia that remained in the culture medium. In free conidia there was also an improvement in this characteristic when the first pulse was applied (24 h).

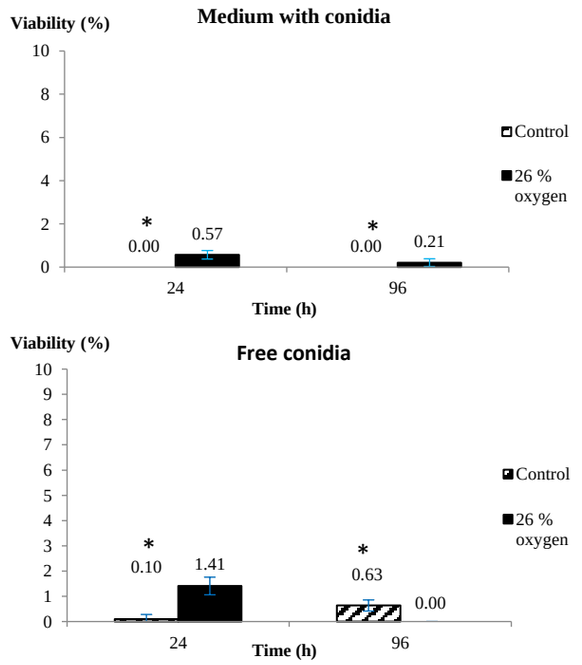


Figure 8 Effect of treatment on high temperature resistance of *Cordyceps fumosorosea* ARSEF3302, *statistically significant difference
Own Source

In the case of *Metarhizium robertsii* Xoch8.1 (Figure 9), it was observed that the first oxidative pulse (24 h) improved the resistance to high temperature in the conidia that remained in the culture medium. Differently, in free conidia this characteristic was improved.

ISSN 2523-6857

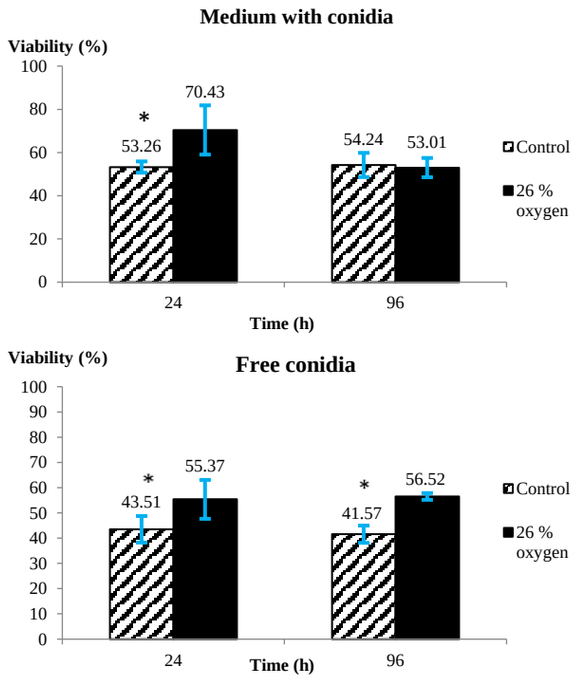


Figure 9 Effect of treatment on high temperature resistance of *Metarhizium robertsii* Xoch8.1, *statistically significant difference
Own Source

2.3 Osmotic stress resistance

In the case of Beauveria bassiana Tac1.1 (Figure 10), it was observed that oxidant pulses enhanced resistance to osmotic stress in conidia remaining in the culture medium and in free conidia.

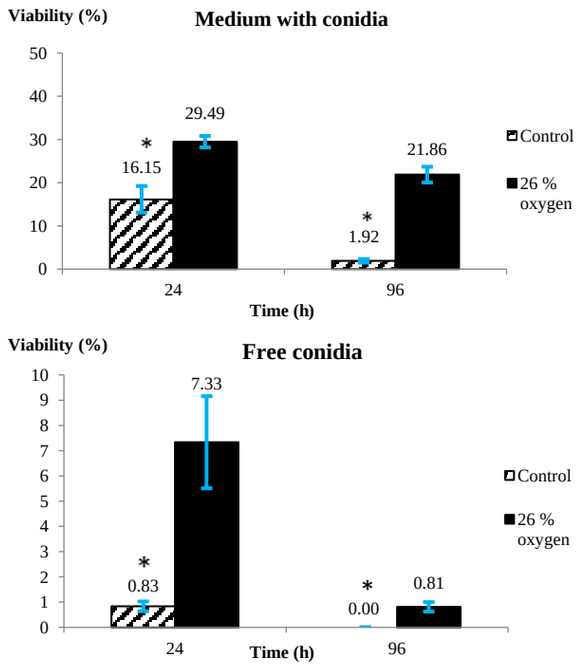


Figure 10 Effect of treatment on osmotic stress resistance of *Beauveria bassiana* Tac1.1, *statistically significant difference
Own Source

In the case of *Cordyceps fumosorosea* ARSEF3302 (Figure 11), it was observed that the oxidative pulses improved the resistance to osmotic stress in the conidia that remained in the culture medium. Differently, the characteristic was not modified in free conidia upon application of the first pulse (24 h), however it was affected with constant treatment.

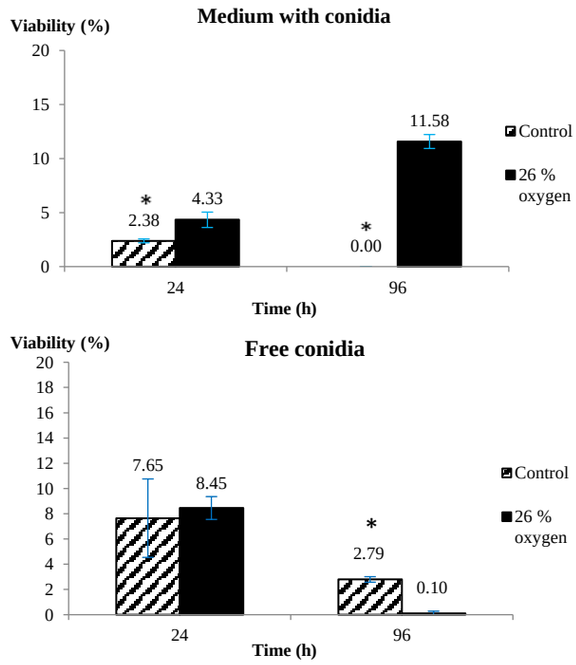
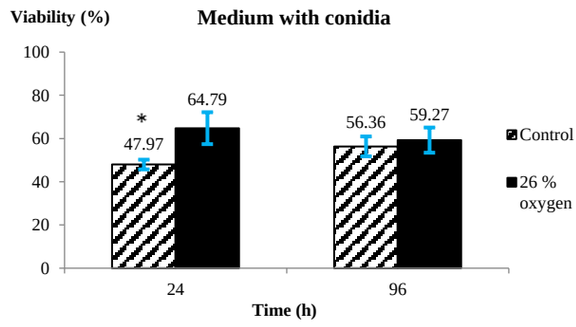


Figure 11 Effect of treatment on osmotic stress resistance of *Cordyceps fumosorosea* ARSEF3302, *statistically significant difference
Own Source

In the case of *Metarhizium robertsii* Xoch8.1 (Figure 12), it was observed that the first oxidant pulse (24 h) improved the resistance to osmotic stress in the conidia that remained in the culture medium. With free conidia, this characteristic was improved.



ISSN 2523-6857

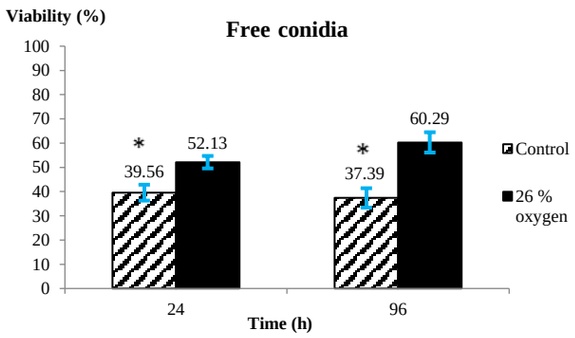


Figure 12 Effect of treatment on osmotic stress resistance of *Metarhizium robertsii* Xoch8.1, *statistically significant difference
Own Source

3. Discussion

Interestingly, it was observed that a single oxidant pulse was sufficient to improve the viability (2.1 section results) of the conidia of *Beauveria bassiana* Tac5.1 and *Metarhizium robertsii* Xoch8.1 fungi in the culture medium; however, with *Cordyceps fumosorosea ARSEF3302* the positive effect was also present, but with more pulses. On the other hand, in the free conidia only *Beauveria bassiana* Tac5.1 showed an improvement of 160 % concerning the control, which was also greater than the improvement of 90 %, which was presented with the conidia in culture medium.

In the case of high heat resistance (2.2 section results), again only one oxidant pulse was sufficient to improve this characteristic in the conidia of the three fungi studied, both in the free state and on culture medium. With free conidia of *Beauveria bassiana* Tac5.1, there was a greater improvement (2.6-fold) compared to conidia in culture medium (1.7-fold). In the case of *Cordyceps fumosorosea* ARSEF3302, due to the low viability, the numerical treatment to quantify the changes presents much uncertainty, however, qualitatively it can be seen that the treatment favors the characteristic in the conidia with medium. In the case of free conidia it was possible to determine a 13-fold improvement, concerning the control. The conidia of *Metarhizium robertsii* Xoch8.1 showed a general improvement of approximately 30 %.

In the case of osmotic stress resistance (2.3 section results), an oxidant pulse caused a positive effect on conidia of *Beauveria bassiana* Tac5.1 and *Metarhizium robertsii* Xoch8.1, both free and in culture medium.

With free conidia of *Beauveria bassiana* Tac5.1, there was a greater improvement (780 %) compared to conidia on culture medium (80 %). *Metarhizium robertsii* Xoch8.1 conidia, maintained the overall improvement of approximately 30 %, including a 60 % improvement when more oxidant pulses were applied to the free conidia. In the case of *Cordyceps fumosorosea* ARSEF3302, an improvement of 80 % was determined with the conidia with culture medium.

These results indicate conidia of the entomopathogenic fungi studied have the ability to respond to the sublethal treatment used in this research. In addition, they can increase their resistance to more than one adverse condition, which is congruent with what has been observed in other research (Miranda-Hernández et al., 2016; Rangel, 2011). It is possible that oxidative pulses lead to the activation of defense mechanisms of the conidia of the fungi studied, for example, Castillo-Minjarez *et al*. (2019) reported the activation of the glutathione system as a non-enzymatic defense mechanism against oxidative conditions. In turn, Garcia-Ortiz *et al*. (2018), studying the proteome of conidia from oxidant atmospheres of *Metarhizium lepidiotae* CP-OAX, found proteins involved in the activation of general stress response mechanisms. However, further molecular studies are needed to understand the response mechanisms, especially of free conidia.

Acknowledgment

The authors would like to gratefully acknowledge the Universidad Autónoma Metropolitana and the Universidad Tecnológica de Tecámac for all the provided support.

Conclusions

This is the first time that the effect of oxidant pulses on the quality of free conidia and conidia in culture medium has been studied simultaneously on entomopathogenic fungi from the same production lot. To determine in which condition there would be the best effect, it is concluded that it is better to apply the treatment on free conidia of *Beauveria bassiana* Tac5.1 and *Metarhizium robertsii* Xoch8.1. On the contrary, in the case of *Cordyceps fumosorosea* ARSEF3302, it is better to apply the treatment to conidia on culture medium.

ISSN 2523-6857

These results may be of interest in the case of seeking to improve the quality traits of the strains studied in their commercial production. However, in order to apply the treatment to other strains, it would be necessary to study the effect, since, as was observed in this work, the response to the oxidant pulse will depend on the genus or species of entomopathogenic fungus to be used.

References

García-Ortiz, N., Figueroa-Martínez, F. J., Carrasco-Navarro, U., Favela-Torres, E., & Loera, O. (2018). The oxygen concentration in cultures modulates protein expression and enzymatic antioxidant responses in Metarhizium lepidiotae conidia. Fungal biology, 122(6), 487-496. https://doi.org/10.1016/j.funbio.2017.10.013

García-Ortiz, N., Tlecuitl-Beristain, S., Favela-Torres, E., & Loera, O. (2015). Production and quality of conidia by Metarhizium anisopliae var. lepidiotum: critical oxygen level and period of mycelium competence. Applied microbiology and biotechnology, 99(6): 2783-2791. https://doi.org/10.1007/s00253-014-6225-2

Méndez-González, F., Castillo-Minjarez, J. M., Loera, O., & Favela-Torres, E. (2022). Current developments in the resistance, quality, and production of entomopathogenic fungi. World Journal of Microbiology and Biotechnology, 38(7), 115. https://doi.org/10.1007/s11274-022-03301-9

Miranda-Hernández, F., Garza-López, P. M., & Loera, O. (2016). Cellular signaling in cross protection: An alternative to improve mycopesticides. Biological Control, 103, 196-203. https://doi.org/10.1016/j.biocontrol.2016.09.007

Miranda- Hernández, F., Saucedo- Castañeda, G., Alatorre- Rosas, R., & Loera, O. (2014). Oxygen- rich culture conditions enhance the conidial infectivity and the quality of two strains of Isaria fumosorosea for potentially improved biocontrol processes. Pest management science. 70:661-666. https://doi.org/10.1002/ps.3605

Rangel, D. E. (2011). Stress induced cross protection against environmental challenges on prokaryotic and eukaryotic microbes. World Journal of Microbiology and Biotechnology, 27(6), 1281-1296. https://doi.org/10.1007/s11274-010-0584-3

Rangel, D. E., Braga, G. U., Fernandes, É. K., Keyser, C. A., Hallsworth, J. E., & Roberts, D. W. (2015). Stress tolerance and virulence of insect-pathogenic fungi are determined by environmental conditions during conidial formation. Current genetics, 1-22. https://doi.org/10.1007/s00294-015-0477-y

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

United Nations (2018). The 2030 Agenda and the Sustainable Development Goals: An opportunity for Latin America and the Caribbean (LC/G.2681-P/Rev.3), Santiago. https://repositorio.cepal.org/items/1a875454-76d9-48fd-a4d2-a0d4526a4c96

ISSN 2523-6857