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Increase in the mortality of the entomopathogenic fungus *Metarhizium anisopliae* due to the application of an electric field during conidiation

Incremento en la mortalidad del hongo entomopatógeno *Metarhizium anisopliae* al aplicar un campo eléctrico durante la conidiación

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Abstract

The effects of an electric field on conidia production by *Metarhizium anisopliae* in solid state culture were determined. A 450-mL electrochemical cell (EC) with titanium ruthenium-oxide coated electrodes and packed with a mixture of rice: corn stubble, was inoculated with 1×10^6 conidia (g of initial dry support)$^{-1}$ and incubated for 8 days (30 ºC). Four days after starting the culture, a current density of 0.09 mA cm^{-2} was applied for 24 h. The moisture kept constant (75%), with and without electric field. With electric field, conidiation ($4.9\times10^8 \pm 3.7\times10^7$ conidia (g of initial dry support)$^{-1}$) did not show statistically significant differences, but their viability and germination (67 and 45%, respectively) were lower than in the non-perturbated conidia. Total mortality of *Tenebrio molitor* larvae provoked by conidia produced in presence of the electric field was 40% higher compared with the control. The study showed that the application of electric field improving the conidial infectivity. This new approach is susceptible to be optimized to other fungi for biological control with the purpose of improving field performance and further investigations on the modification of cellular mechanisms by the electric field stimuli.

Keywords: Electric field, solid-state culture, entomopathogen fungus, mortality.

Resumen

Se determinaron los efectos de la corriente eléctrica sobre la producción de conidios de *Metarhizium anisopliae* en cultivo sólido. Se usó una celda electroquímica con electrodos de oxido de titanio y rutenio y como sustrato una mezcla de arroz: rastrojo de maíz, el cual fue inoculado con 1×10^6 conidios (g de soporte seco)$^{-1}$ e incubado por 8 días (30 ºC). Cuatro días después de iniciado el cultivo, se aplicó una densidad de corriente de 0.09 mA cm^{-2} durante 24 h. La humedad se mantuvo constante (75%), con y sin campo eléctrico. En ausencia y presencia del campo eléctrico, la conidiación ($4.9\times10^8\pm3.7\times10^7$ conidias (g de soporte inicial seco)$^{-1}$) no mostró diferencias significativas, pero la viabilidad y germinación (67 y 45%, respectivamente) fueron menores respecto al control (sin campo eléctrico). La mortalidad en larvas de *Tenebrio molitor* por conidios producidos con campo eléctrico fue 40% mayor que en los conidios control. Este estudio mostró que la aplicación del campo eléctrico mejora la infectividad conidial. Este nuevo enfoque es susceptible de ser optimizado para otros hongos usados en control biológico con el propósito de mejorar el rendimiento en campo y futuras investigaciones sobre la modificación de los mecanismos celulares por los estímulos del campo eléctrico.

Palabras clave: Campo eléctrico, cultivo en medio sólido, hongo entomopatógeno, mortalidad.

1 Introduction

The lack of knowledge and misuse of chemical pesticides in agriculture has caused an imbalance in the ecosystem, affecting human health and the appearance of resistance in many species of insects (Ndiath, 2019). In this way, biological control is a sustainable, ecological tool for pest control, in which only the pathogenic microorganism is adversely affected, promoting the development of healthy crops and causing the least possible damage to agroecosystems (Safavi *et al*., 2007).

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In this context, the entomopathogenic fungus (EF) *Metarhizium anisopliae* (Ascomycota: Hypocreales) have been widely used for biological control of pests that colonize plant species from different ecosystems, some with agricultural importance like banana, cocoa, coffee, among others (McGuire and Northfield, 2020). The EF are worldwide distributed and have a spectrum of control that includes egg, adults and larvae of insects; such as moths, crickets, ants, beetle, weevils, grasshoppers, thrips, aphids, mites and many others (Araújo and Hughes, 2016).

Due to its infective cycle, the propagation unit is the conidium, which should adhere to insect cuticle and together with the production of appresorium and enzymes penetrate the insect, reproduces inside, causing death related to a general metabolic deterioration produced by secondary metabolites, among others mechanisms. Once death is produced, the mycelia emerges on the surface of the insect in order to propagate its infective unit in a natural manner. Therefore, the integrity of the conidium and its quality parameters such as conidial germination, viability and degree of infectivity are main issues to be evaluated together with the conidia production (Miranda-Hernández *et al*., 2014). An initial and rapid assessment through laboratory bioassay using *Tenebrio molitor* (Coleoptera: Tenebrionidae), a model insect susceptible to some EF (Sáenz-Mendoza *et al*., 2019), can be a strategy to evaluate the holistic performance of the conidia related to their production conditions (Rodriguez-Gomez *et al*., 2009).

Initial approaches to increase the conidia production at an industrial level have been the modification of substrates or the culture conditions (Lopez-Perez *et al*., 2015). Rodriguez-Gomez *et al*. (2009) evaluated different substrates in surface culture for conidia production of *Beauveria bassiana*, finding the highest production on Sabouraud dextrose agar, and maintaining a high degree of virulence on *Tenebrio molitor*. Another strategy was the use of support material to increase the porosity of the packing bed in solid state cultivation of *Isaria fumosoroseae* that may increase oxygenation and conidiation even at 100% (Angel-Cuapio *et al*., 2015) without affecting virulence.

Recently, different strategies have been investigated, some of them related to increase the stress conditions, by changing temperature, moisture, atmosphere on some EF cultivation, such as, *Metarhizium anisopliae*, *Beauveria bassiana* and *Isaria fumosorosea*. Tlecuitl-Beristain *et al*., (2010) increased the concentration of oxygen in the atmosphere, increasing the conidia production of *Metarhizium anisopliae* var. *lepidiotum*, up to 2.6 times more than the control, without altering the virulence on adult *Tenebrio molitor*. Light regimes may also generate some kind of stress related to cell differentiation induction in EF like *Metarhizium robertsii* (Oliveira *et al*., 2018). In this way, it is necessary to find new stress strategies that allow increasing both conidia production and virulence.

On the other hand, electric fields can provoke an oxidative stress associated to the formation of oxygen reactive species (Nezammahalleh *et al*., 2016). The application of low intensities (0.42 mA cm^{-2}) of electric perturbation during the cultivation modifies the metabolism in filamentous fungi like *Aspergillus niger* (Velasco-Alvarez *et al*., 2011), in this way, this modification could be attributed to transitory increases in the permeability of mobile membranes, as well as stimulation in CO_2 production, O_2 consumption and conidia overproduction (Velasco-Alvarez *et al*., 2017). After these previous studies, the electric field was proposed as stress condition to modify the metabolism of EF toward increasing conidia production and field performance. The aim of this study was to evaluate the effect of the electric field on the *Metarhizium anisopliae* conidia production in solid culture and the quality parameters of the obtained conidia, focused on their infectivity, including mortality against larvae of *Tenebrio molitor*.

2 Materials and methods

2.1 Microorganism, strain conservation and propagation

The fungal strain *Metarhizium anisopliae* var. *lepidiotum* (CP-Oax) (Postgraduates College Collection, Texcoco, Mexico), corresponds to the identification number ENCB-MG-81 into the Culture Collection ENCB-IPNWDCM449 (Molecular identification of this strain was performed by Tlecuitl-Beristain *et al*., 2010). The inoculum was obtained by propagating the fungus in Erlenmeyer flasks (250 mL) containing oat-peptone agar medium (33.3, 10 and 15 g L^{-1}, respectively) for 7 days (30 °C), and conidia were harvested by using a Tween 80 solution (0.5%). For preservation, 1 cm^2 of 7-days old mycelial squares of the fungus grown on potato-dextrose agar (PDA) medium were immersed in glass vials with sterile distilled water (4 °C).

2.2 Substrate

The corn stubble was ground, dried and sieved (10 mesh) to obtain a particle size between 0.66 and 0.77 mm in diameter. Parboiled rice (Valle Verde®) was used. A mixture of parboiled rice: corn stubble in a 9:1 ratio was used as the organic support for the solid state culture. The mixture was hydrated with distilled water (3.3 mL g of dry rice^{-1}) during 24 h before sterilization. The pH of the mixture was adjusted to 5 with 0.1 mol L^{-1} HCl, later the mixture was autoclaved at 120 ºC for 15 min.

2.3 Electrochemical cell characterization

A cylindrical acrylic cell (450 mL: 18 cm long and 6.5 cm in diameter) with two lateral containers for the electrolytic solutions (0.1 M KH2PO4) and titanium electrodes coated with ruthenium (14.13 cm^2) was used (Velasco-Alvarez *et al*., 2011). These types of electrodes are known to favor water oxidation/reduction reactions and exhibit a high corrosion resistance (Trasatti, 2000). The electrochemical cell (EC) was packed with the rice: corn stubble mixture (9:1) previously moisturized (75%), reaching full contact of the electrodes with the support. Electric current was controlled with a power source (EXTECH 382200). During electric current application, cell potential (Ecell V-1) was measured with a PROTEK 506 Digital multimeter (high impedance), obtaining a plot of current density (mA cm^{-2}) vs. Ecell V-1. The electrochemical cell characterization was performed by applying different electric current (0.1-50 mA) to the inside electrodes; the cell potential was measured for each current. The appropriate range of electric current intensity was chosen in the range of ohmic behavior and where the temperature was not changed inside the EC.

2.4 Culture conditions

The EC was packed with 28 g of the sterile mixture of rice: corn stubble, 270 μL of 0.5% chloramphenicol were added. Subsequently, it was inoculated with a suspension of 1×10^6 conidia per gram of initial dry support (gids) and incubated for 8 days (30ºC). After four days of culture, in the presence of mycelium, the electric current was applied using a power source (EG & G, PARC 173) during 24 hours (1.3 mA; 0.09 mA cm^{-2}), then the electric current was disconnected and the culture continued for three more days (Velasco-Alvarez *et al*., 2011). In order to measure response variables, the EC was divided into three equidistant longitudinal sections between anode and cathode (4 cm per section). The following response variables were measured in triplicate for each section: pH, moisture (%), production of conidia, viability, germination and mortality. Measurements were made on cultures with and without exposure to electric current on 4, 5, 6, 7 and 8 days after the start of the culture. At each time point, the whole EC was sacrificed, and for the subsequent samplings a newly prepared culture was used. Each time represents independent experiments performed in triplicates.

2.5 Moisture and pH

Moisture in the samples were directly determined in an Ohaus Thermo scale (model MB45). The sample (1.5 g) was placed in aluminum trays, and the temperature was increased to 110 °C until constant weight was obtained. To determine the pH, one gram of sample was suspended in 9 mL of distilled water, the suspension was stirred for 5 minutes. The pH reading was recorded in the supernatant with a digital potentiometer (Conductronic pH 20). The moisture and pH were made on cultures with and without exposure to electric current on 0, 4, 5, 6, 7 and 8 days.

2.6 Conidia production and viability

Conidia was quantified by suspending 2 g of fresh sample in 9 mL of 0.05% Tween 80 solution and gently stirred. The conidia count was done in a Neubauer chamber, and production levels were reported as conidia per gram of initial dry support (conidia gids^{-1}). To determine viability, a conidial suspension was adjusted to a concentration of 1×10^4 conidia per mL, and 30 μL were inoculated in Petri dishes with Sabourad dextrose agar (SDA) medium. Colony forming units were counted after 72 h of incubation at 28 ºC.

2.7 Germination

A sterilized square of water-agar (1.5%) was placed on a slide, and it was inoculated with 100 μl of a conidia suspension (1×10^6 conidia per mL). The slide was placed on a wet paper filter inside a sterile Petri dish at constant temperature (28 ºC) for 7 h (Miranda-Hernández *et al*., 2014). Then, microscopic observations (Olimpus, 40x) of at least 100 conidia were done; a germinated conidium was considered when the germ tube was equal or greater than its width (Safavi *et al*., 2007).

2.8 Mortality of Tenebrio molitor larvae

To determine the mortality, suspensions of 2×10^7 conidia per mL harvested after 8 days of the treatment with electric current and control without electric current application were used, (Rodriguez-Gomez *et al*., 2009). Experimental units were Petri plates containing 12 larvae of *Tenebrio molitor* and having oat flakes as food. The immersion time in the conidia suspension was 10 s. A Tween 80 solution (0.01%) without conidia was used as control for bioassays. The plates were kept at 28 °C, with a photoperiod of 12 h. Three experimental units were used per treatment; they were enclosed in a plastic container with a moisturized filter paper. Mortality was recorded daily and dead insects were transferred to moist chambers to encourage external sporulation in order to confirm that death was due to fungal infection. Survival percentage was registered and specific parameters were estimated according to the previously proposed model (Rodriguez-Gomez *et al*., 2009).

2.9 Statistical analysis

Data were analyzed using NCSS-2000 software, version 2001 (Copyright 2001 by Jerry Hintze). The analysis of variance (ANOVA) was performed with $\alpha < 0.05$ and different groups were obtained by Tukey test.

3 Results and discussion

3.1 Characterization of the Electrochemical Cell

The characterization of the electrochemical cell in abiotic conditions was carried out in order to establish the operation parameters avoiding temperature changes or chemical variations during the application of the electric field that would affect the growth and development of *Metarhizium anisopliae*. The operating values of the EC were established applying different current intensities (0.1-50 mA) between the electrodes and the support (anode-rice: corn stubble-cathode).

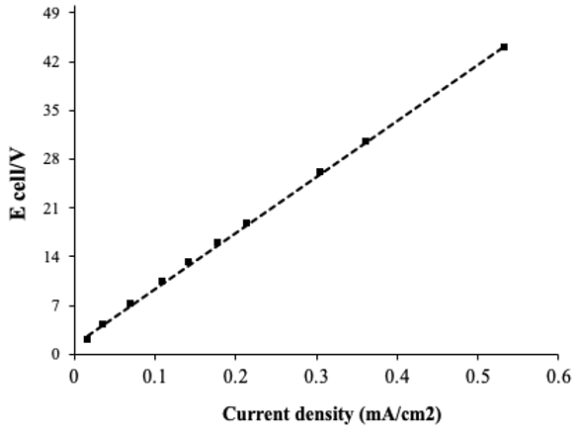

Fig. 1. Cell characterization in abiotic conditions. Variation of cell potential (E cell V^{-1}) as a function of the imposed electric current density (mA cm^{-2}). Electrochemical cell packed with rice: corn stubble previously moistened with distilled water. Error bars represent deviations of three replicates for all assays.

The cell potential (V) linearly increases as the applied current intensity was increasing (Figure 1). The registered cell potential was 45 V (Ecell V^{-1}) when 8 mA was applied (0.53 mA cm^{-2}); despite the higher potential in the cell an apparent constant temperature inside the EC (30 ºC) was registered. This contrasts with the results previously reported by Velasco-Alvarez *et al*., (2017), where an EC packed with a porous support (perlite) registered cell potentials of 20 V (Ecell V^{-1}) for a 50 mA (3.5 mA cm^{-2}) applied current. This indicates that the support used in the present study (rice: corn stubble) is highly resistive to the passage of electric current, which could be associated with the low conductivity and the low availability of free water from the support. Rice has a high capacity to absorb water (2.8 mL g of $rice^{-1}$), and the amount of free water to carry out the ionic conduction is low. On the other hand, corn stubble that was used to increase porosity and improve O_2 transfer through the support also contributed to resistivity, due to the air gaps formed by the rice and corn stubble (Angel-Cuapio *et al*., 2015). Therefore, based on these results and considering previous studies where it has been shown that at intensities above 20 V the metabolism of fungi can be inhibited (Velasco-Alvarez *et al*., 2011), a current density of 0.09 mA cm^{-2} (1.3 mA) was selected according to electrode surface (14.13 cm^{-2}) to develop a cell potential of 10 V for this study.

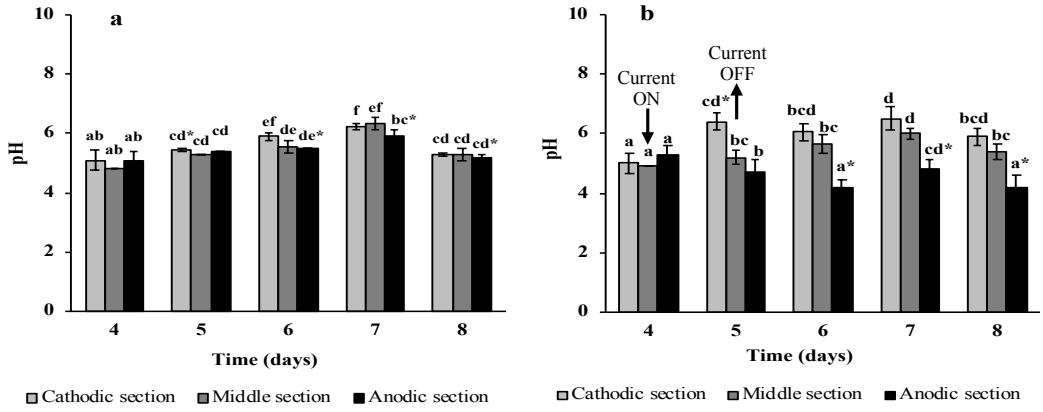

Fig. 2. Changes in pH values of the cathodic, middle and anodic sections of the electrochemical cell. (a) control without electric current, (b) treatment with electric current (24h: 1.3 mA). Each bar represents the mean value of three independent assays with the corresponding standard deviation. Letters mean statistically significant differences within treatment (comparing days and sections), while asterisks mean statistically significant differences between treatments (a *vs*. b).

3.2 Moisture and pH

The application of the electric current on a solid state culture system demands to control the relative moisture in the system in order to allow a convenient passage of ionic conduction, without having an increase in temperature or cell potential variations. The moisture remained constant over time in the three sections of the cell with and without an electric current (74-76%). Although the high moisture content could compromise conidial production, the presence of corn stubble was used to increase the porosity in the system allowing increased availability of substrate and oxygen, which is also an important point when growing in a solid state system (Simas-Dias *et al*., 2018). Therefore, a balance of the cell characterization and moisture results are needed in order to obtain an increase in conidiation. Figure 2 shows the pH variations during the 8 days of culture. The pH of composites was adjusted to 5 before sterilization, and in the control cells (without electric current) did not show statistically significant differences, maintaining a pH between 5.5 ± 0.4 (Figure 2a).

On the other hand, the cultures exposed to the electric field during 24 h after 4 days of incubation showed variations on pH in the three different sections (Figure 2b). There were no statistically significant differences between the cathodic and middle sections (5.9 ± 0.4), nevertheless, significant changes on pH were found when compared to the anodic section, which showed a lower pH (4.4 ± 0.3). It is known that during the application of an electric field, electrolysis of free water takes place to assure the transport of charge between the electronic conductors and the support, producing an acid front near the anode and an alkaline front near the cathode, when both fronts meet at the middle section, they neutralize each other (Acar and Alshawabkeh, 1993). This behavior is mainly due to the transfer of charges between the support and the electronic conductors (electrodes) achieved through electrochemical water oxidation, which depending on the availability of the chemical species and the electrochemical potential present in the medium, these ions may be transported through the support. Therefore, the pH gradient depends on the availability of water and the relative mobility of hydronium and hydroxyl ions through the support (Pamukcu and Wittle, 1993) that is, the greater the resistance, the less movement of the ions throughout the support and consequently pH gradients are less drastic, as observed in this study. Despite the pH gradient observed during the application of the electric field, the pH values remained between the optimal values (4.5 - 6.2) for the growth and conidia production by *Metarhizium anisopliae* (Chandra Teja and Rahman, 2017).

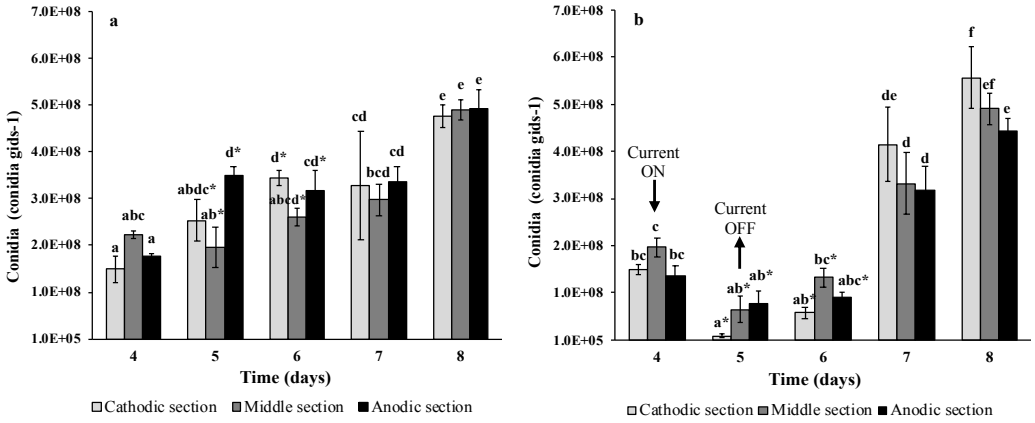

Fig. 3. Conidia production on rice: corn stubble in the cathodic, middle and anodic sections of the electrochemical cell. (a) control without electric current, (b) treatment with electric current (24h: 1.3 mA). Each point represents independent assays; error bars represent deviations of three replicates for all assays. Letters mean statistically significant differences within treatment (comparing days and sections), while asterisks mean statistically significant differences between treatments (a *vs.* b).

3.3 Conidia production

Figure 3 shows the kinetics of conidia production of *M. anisopliae* var. *lepidiotum* in the presence and absence of the electric field along the three sections of the electrochemical cell. The conidia production during the first four days of culture in the electrochemical cell was $1.7\times10^{8}\pm 3.3\times10^{7}$ conidia gids-1. In the control cell (without an electric current), a gradual and homogeneous fungal growth was observed, quantifying at the end of the culture (8 days) $4.9\times10^{8}\pm 9.3\times10^{6}$ conidia gids-1 (Figure 3a). In the electrochemical cells in which an electric field was applied after 4 d of culture, an inhibition (up to 5 times less production than in the control) in the conidia production was registered during the following 2 days (at 5 and 6 days of culture). Despite this inhibition, at the end of the culture (8 days) no statistically significant differences were observed in the production of conidia between the cells with and without an electric current (Fig. 3b). On the other hand, for the EC with electric current applied, a smaller conidia production ($4.5\times10^{8}\pm 1.43\times10^{7}$) was observed in the anodic section after 8 days. The great heterogeneity in the conidia production depending on the section of the EC might be related to the observed pH changes. We can also suggest that the positive effect of the current was only observed after seven days of cultivation and in a single section of the EC (cathode).

It has been proposed that the application of an electric current increases the formation of reactive oxygen species (ROS) and oxidative stress. When ROS come into contact with cells, membrane potential, ion concentration (Nezammahalleh *et al*., 2016), as well as cell differentiation can be affected (Aguirre *et al*., 2005). Velasco-Alvarez *et al*., (2017) found that the application of low intensities of electric current, as well as the pH gradient generated causes a hyperoxidative state of stress (measured as the accumulation of malondihaldehyde) that can be associated to an inhibition of biomass production. Therefore, our results suggest that the electric current only had a transient inhibition effect measured as conidia production 48 h after the electric current was disconnected, in spite of such an inhibition, at the end of the culture no statistically significant differences in conidia production were observed between control and treatment.

3.4 Germination and viability

Germination and viability of conidia are determining parameters of quality, to evaluate the efficiency of an EF, particularly when applied against larvae.

The germination (in percentage) of the conidia produced in the different sections of the cell are shown, in the absence (Figure 4a) and presence of the electric field (Figure 4b).

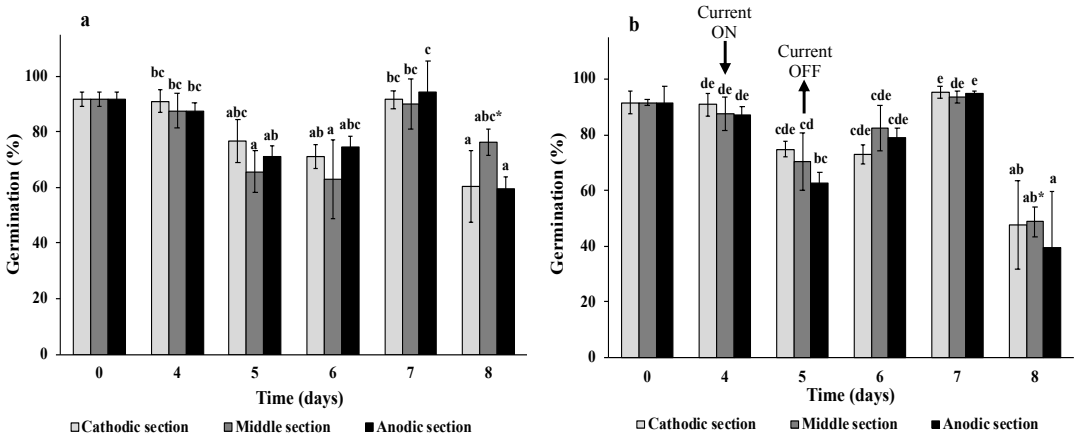

Fig. 4. Germination of conidia on rice: corn stubble in the cathodic, middle and anodic sections of the electrochemical cell. (a) control without electric current, (b) treatment with electric current (24h: 1.3 mA, after 4th day). Germination analysis were performed after 7 h of culture. Each bar represents the mean value of three independent assays with the corresponding standard deviation. Letters mean statistically significant differences within treatment (comparing days and sections), while asterisks mean statistically significant differences between treatments (a *vs.* b).

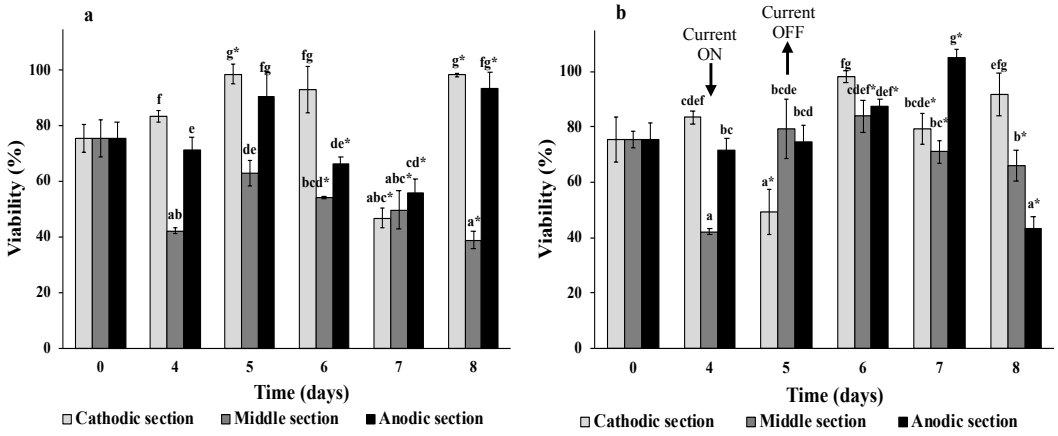

Fig. 5. Viability of conidia harvested from rice: corn stubble in the cathodic, middle and anodic sections of the electrochemical cell. (a) control without electric current, (b) treatment with electric current (24h: 1.3 mA, after 4th day). The number of colony forming units were determined by plate count in SDA medium. Each bar represents the mean value of three independent assays with the corresponding standard deviation. Letters mean statistically significant differences within treatment (comparing days and sections), while asterisks mean statistically significant differences between treatments (a *vs.* b).

The germination percentage remained between 50 and 90% throughout the culture. Only at the end of the culture (8 days), no statistically significant differences were observed among the anodic, middle and cathodic sections. However, differences between absence or presence of electric field was only observed in the middle section, been 27% higher (76 ± 5) in the absence than in the electric field treatment. Although germination is a response variable to evaluate the capacity of EF when used as a biological control strategy, it is also important to determine their viability, since a germinated conidium will not necessarily produce a colony, which is essential to initiate the infective cycle on the pest (San Aw and Hue, 2017).

Figure 5 shows the viability results (%) with respect to time, of conidia harvested from the EC in presence and absence of the electric field, and counted as colony forming units after 72 h of cultivation at 30 ºC. For those conidia harvested the 5th day from the EC exposed to electric field a decrease (20%) in the conidia viability was registered with respect to the control. At 7 days, a statistically significant difference was observed in conidia coming from the cell with electric field applied (85 ± 16%) compared to the that obtained in the control EC (51 ± 6%). According to literature, changes in viability of conidia could be related to different nutritional and environmental factors such as temperature, light and pH. However, since in this study, there were no changes in any of the above variables, which could affect the development of conidia, it is possible to ensure that the observed effects are only due to the application of the electric field.

When analyzing the germination and viability results (Figures 4, 5) it was observed that the average percentage of germination remained between 75 to 91% during the first 7 days of culture, both in treated and untreated conidia; however, the viability presented a lower percentage than that observed in germination (59-77%). In other words, almost 20% of the germinated conidia were unable to develop a colony-forming unit. The determination of these parameters in the produced conidia is of great importance, not only because these quality parameters allow predicting their behavior in field, but also because it can give a clue on the effect of the electric current on the integrity of the obtained conidia with this treatment.

3.5 Infectivity bioassays

The infectivity of the conidia produced after eight days of culture at the three sections of the EC with and without exposure to electric current was tested in *Tenebrio molitor* larvae, results are shown in Figure 6.

After four days of the bioassay, a significant increase (29 ± 6%) was observed in the mortality of the larvae exposed to conidia produced with an electric field, compared to the control conidia (11 ± 3%). At the end of the bioassay (14 days) the highest percentage (88%) of accumulated mortality was found in the experimental units containing larvae infected with conidia exposed to an electric field, compared to the controls (50%). When comparing by sections of the electrochemical cell, those conidia grown in the cathodic and middle sections reached 93 ± 2% mortality, it was significantly different for those harvested from the anodic section (78 ± 5%), meanwhile the conidia harvested from the control only reached 53 ± 5%. The mortality observed in Tween solution reached 28 ± 5% by day 14 of assay, it is worth to mention that it was zero until day ten of assay, when treatment curves had reached the highest value of accumulated mortality. The application of the electric current improved the characteristics of the conidia in terms of mortality, since the time (4.87 d ± 0.12) to kill 50% of the larvae population (TL_{50}) was lower compared to the respective control (10 d ± 1.7). In addition, other infectivity parameters, such as t_0, which is the time when mortality begins, was similar in all treatments (~3 d); however, the *k* (speed of death) was significantly higher (0.6 ± 0.16 d^{-1}) in the treatment with electric current compared to control (0.25 ± 0.12 d^{-1}).

Table 1 summarizes the infectivity results together with the response variables of the conidia harvested on the last day of cultivation (8 days).

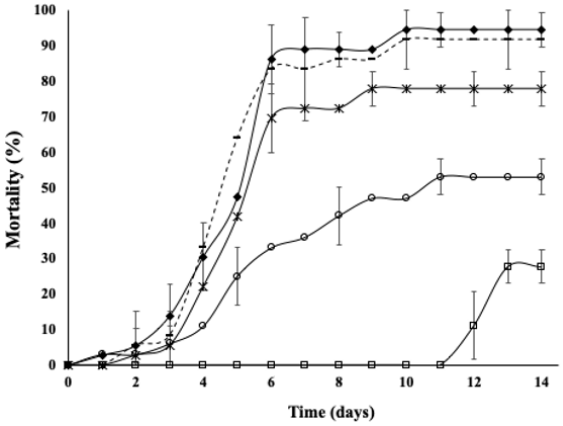

Fig. 6. Mortality of Tenebrio molitor larvae after 14 days of being exposed to conidia harvested from rice: corn stubble. Data obtained in the presence of the electric field; (-◆-) cathodic section, (- - -) middle section, (-∗-) anodic section. In absence of the electric field; (-o-) control, (-□-) Tween solution. Each bar represents the mean value of three independent assays with the corresponding standard deviation.

Table 1. Response variables of the conidia harvested after 8 days of culture in the presence or absence of the electric current (0.09 mA cm^{-2}: 24 h, after 4th day).

Variables	Anodic section		Middle section		Cathodic section	
	Current density (0 mA cm^{-2})	Current density (0.09 mA cm^{-2})	Current density (0 mA cm^{-2})	Current density (0.09 mA cm^{-2})	Current density (0 mA cm^{-2})	Current density (0.09 mA cm^{-2})
pH	5.2 ± 0.1 [a]	4.2 ± 0.4* [b]	5.3 ± 0.2 [a]	5.4 ± 0.3 [a]	5.3 ± 0.1 [a]	5.9 ± 0.3 [a]
Conidia ($gids^{-1}$)	$4.9x10^8$ ± $4.0x10^7$ [a]	$4.4x10^8$ ± $2.6x10^7$ [a]	$4.9x10^8$ ± $2.2x10^7$ [a]	$4.9x10^8$ ± $3.4x10^7$ [a]	$4.8x10^8$ ± $2.5x10^7$ [a]	$5.6x10^8$ ± $6.6x10^7$ [a]
Viability (%)	94 ± 6 [a]	43 ± 4* [a]	39 ± 3 [b]	66 ± 6* [b]	98 ± 1 [a]	92 ± 8 [c]
Germination (%)	60 ± 4 [a]	48 ± 16 [a]	76 ± 5 [b]	49 ± 5* [a]	60 ± 13 [a]	40 ± 20 [a]
Mortality[ψ] (%)	53 ± 5*	78 ± 5* [a]	53 ± 5*	92 ± 8* [b]	53 ± 5*	94 ± 5* [b]

[Ψ]Acumulated mortality expressed as the dead larvae (%) after 14 d of bioassay. Different letters indicate significant differences among the cathodic, middle and anodic section for each cell. (∗) means statistically significant differences between the treatment (electric field applied during conidia production) and control.

The conidia grown in absence of an electric field showed no statistically significant differences among the three sections for all the evaluated variables, except for the viability of the middle section. This work demonstrated the positive effect of the low intensity electric current applied during the conidia production of *Metarrizium anisopliae* in solid culture, on the mortality of larvae of *Tenebrio molitor*. The mechanisms by which pathogenicity increased due to the electric field are unknown; however, a modification of conidia surface properties with the electric potential could be associate to this positive effect. It is well known that the surface properties of fungal cells are the basis for the host-pathogen interaction (Ting-Ting and Ming-Guang, 2011) and that some increase in conidial hydrophobicity may be related to increase in the initial adhesion to the insect cuticle. Holder *et al*., (2007) propose that the surface charges of the conidia, quantified through the zeta potential, present positive charge values at pH 3 (+ 22 ± 2 mV), and that it can rapidly change to a negative charge when the pH rises above 8-9 (- 230 ± 4 mV); and that the increase in the infectivity of the conidia is associated with the increase in hydrophobicity and zeta potentials. However, in our case no drastic changes in pH were observed, since the ion conduction in our system was very low, so we can assure that the mortality modifications of the conidia are associated only with the electric field.

A hydrophobicity study could give sustenance to such a hypothesis. However, this study is a first approach to select among the three sections of an electrochemical cell, the ideal conditions for the production of conidia of entomopathogenic fungi, considering the response variables: germination, viability, pH, conidia production and infectivity of the conidia under the influence of an electric field. Our results showed that the best operating conditions of the EC under the influence of the electric field was the cathodic section, where a high percentage of viability of the conidia (92%) and a high percentage of infectivity was observed on larvae of *Tenebrio molitor* (94%) (Table 1).

Some other quality parameters should also be included in additional analysis, such as performance under field and shelf life conditions, especially with regard to increased resistance to UV light, osmolarity, or temperature changes, as some recent studies have stated that cross talk, which is the acquired resistance to a set of factors when the conidiation occur under some stressful condition, can improve certain quality of the conidia (Rangel *et al*., 2008).

Conclusions

The conidia of *Metarhizium anisopliae* produced after the application of a controlled low intensity electric current density (0.09 mA cm^{-2}: 24 hours, after 4th day) had a positive effect on the mortality of *Tenebrio molitor* larvae. Despite registering some loss of viability and germination, the conidia were able to be more pathogenic toward *Tenebrio molitor* larvae, a model insect, probably caused by alterations in the surface of the conidia. This study is a first approach that allows selecting the ideal operating conditions for an EC in order to improve the production of more infectious conidia of entomopathogenic fungi, under the influence of an electric field.

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Dedication

This work is dedicated to the memory of Dr. Mariano Gutiérrez Rojas, the poet of science and eternal lover of the Moon.

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