Article

Effectiveness of entomopathogenic fungi on the mortality of *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) under laboratory conditions

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Abstract

Currently, at a national and international level, *Dactylopius opuntiae* Cockerell (Hemiptera: Dactylopiidae) has become the main phytosanitary enemy of nopal (*Opuntia* spp.). For their control, there are few options, but the use of agrochemicals has been privileged, with the collateral effects that its use implies. Considering that the use of entomopathogenic fungi represent a viable alternative in the integrated management of this phytophagous, in this investigation the pathogenicity and virulence of three species and two isolates were evaluated: *Beauveria bassiana* (GHA, Bb1), *Metarhizium anisopliae* (Ma129, Ma130) and *Lecanicillium lecanii* (974, 2009) on mortality of second instar nymphs of *D. opuntiae*. The results showed that all isolates evaluated have a different degree of pathogenicity; however, *M. anisopliae* and *L. lecanii* were more effective. Isolation Ma 130, belonging to *M. anisopliae* showed the highest virulence determined in function of the CL_{50} with a value of 6.63 x 10^7 conidia mL^{-1}, under controlled conditions. Therefore, this isolation could be considered in a control strategy of *D. opuntiae*.

Keywords: biological control, nopal, plague.

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Introduction

The cochineal *Dactylopius opuntiae* Cockerell (Hemiptera: Dactylopiidae) is considered the main phytosanitary problem of nopal (*Opuntia* spp.) in Mexico and other parts of the world, due among other aspects to: the presence of a waxy secretion, which provides females camouflage, resistance to adverse climatic factors, protection against natural enemies and reduces the effectiveness of the action of insecticides; the high reproduction rates and short life cycles and the presence of carminic acid, which has antifeedant and deterrents properties.

D. opuntiae is one of the 11 species belonging to the Dactylopiidae family (Spodek *et al*., 2014), which is distributed in 18 countries around the world (García *et al*., 2016). It has been registered causing serious damage in the Valencian community, Spain (Rodrigo *et al*., 2010) and recently its presence has been detected for the first time in Israel (Spodek *et al*., 2014) and in Morocco (Bouharroud *et al*., 2016). Likewise, due to the incidence and severity of the damage, during the last decade it has become the most important pest in nopal fodder plantations in northeastern Brazil (Da Silva *et al*., 2010; Falcao *et al*., 2013). In Mexico, it is present in all nopal zones, located in 22 states of the country (Chávez-Moreno *et al.*, 2011), which gives an idea of its wide distribution and ease of dispersion.

Traditionally, its control has been based on the use of pesticides, among which, malathion, methyl parathion and trichlorfon (Badii and Flores, 2001), although there are currently no authorized insecticides in Mexico to be used in this insect (Vanegas-Rico *et al*., 2010). Derived from the above, the application of unauthorized products or in the doses not recommended may cause risks to consumers, producers and damage to the environment, as highlighted in the report made by the Department of Public Health of the State of California (CDPH), which warns about the danger of consumption of nopal from Mexico in the presence of monocrotophos (5.8 ppm) an organophosphate pesticide banned since 1989. http://www.cdph.ca.gov/Pages/NR14-021.aspx.

In view of this problem, several strategies have been tried and developed in Mexico to combat *D. opuntiae*; through, of alternative methods. For example, Mena and Rosas (2007) recommend brushing or mechanical sweeping of the cladodes. Also, biodegradable products have been evaluated (Palacios-Mendoza *et al*., 2004), plant extracts (Vigueras *et al.*, 2009), essential oils (Vázquez-García *et al*., 2011) and organic and inorganic silicon aspersion mixed with biodegradable soaps (Mena, 2013), among others; however, in all these methods successes will depend on the stage of development in which the insect is found.

Another promising alternative to management has been the use of natural enemies, as agents of biological control (Vanegas-Rico *et al*., 2010). Some of them have been evaluated against *D. opuntiae* such as: *Sympherobius barberi* (Pacheco-Rueda *et al*., 2011), *Chilocorus cacti*, (Flores *et al*., 2013) and *Hyperaspis trifurcata* (Ramírez *et al.*, 2013), among others. However, many of these studies have been restricted to the laboratory phase, so they have not yet been transferred to producers and valued in field conditions. Another aspect little studied in the country is the selection of cultivars resistant to *D. opuntiae*, but in Brazil there are already selection and improvement programs for this purpose (Da Silva *et al*., 2010).

The use of biopesticides, including those formulated with entomopathogenic fungi, are becoming important as an alternative to the use of insecticides. In this aspect, Brazil has highlighted the studies with *B. bassiana* for the control of *D. opuntiae* (Santos *et al*., 2011) and *Fusarium incarnatum-equiseti* (Da Silva *et al*., 2016) but they do not exist, according to our knowledge, studies in Mexico that address the use of different strains of fungi for their control. Therefore, it is intended to evaluate the pathogenicity and virulence of three species of entomopathogenic fungi (*Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii*) on the mortality of second instar nymphs of the cochineal *D. opuntiae*.

Materials and methods

The experiment was carried out in the Insect Pathology Laboratory of the *Campus* Montecillo Postgraduate College, during the period from August 2015 to April 2016.

Obtaining colonies of *D. opuntiae*

The initial breeding foot was obtained through the collection of cladodes infested with *D. opuntiae*, during the month of July 2015 of a commercial plantation for the production of prickly pear, located in the community of Cuautlacingo, Otumba, State of Mexico. The cladodes containing the colonies of *D. opuntiae* were carefully cut, revised to avoid the presence of any natural enemies and subsequently moved and confined in a breeding unit of the Campus Montecillo Postgraduate College, according to the system proposed by Aldama and Llanderal (2003).

Cladode infestation

To obtain cohorts of *D. opuntiae*, once the adult females began reproduction, they were carefully collected and introduced into 'tulle nests', according to the method described by Aldama and Llanderal (2003). Later, cladodes of nopal *Opuntia ficus-indica* (L.) Mill. containing the nests were placed in transparent plastic containers: 25 x 15 x 5 cm long, wide and high, respectively, and introduced into a bioclimatic chamber (Thermo Scientific) at a temperature of 25 ±1 °C and a photoperiod of 24 h dark, for a period of 72 h, in order to ensure the establishment and fixation of the migrating nymph. Once sessile nymphs were obtained, 5 x 5 cm cladodes were cut, in which 30 nymphs were counted. Each unit was placed in 6 x 4 cm transparent cylindrical disposable containers. 13 experimental units were established for each repetition.

Cultivation of isolations used

To carry out the study, the following isolates were used: *Beauveria bassiana* (GHA, Bb1), *Metarhizium anisopliae* (Ma129, Ma130) and *Lecanicillium lecanii* (974, 2009), all of them monosporic, from the collection of entomopathogenic fungi from the Laboratory of Insect pathology of the Phytosanitary Institute of the Postgraduate School (Table 1). The production of conidia was carried out by means of surface culture of solid medium (Butt and Goettel, 2000) using dextrose sabouraud agar (BD Bioxon, Mexico) in the following proportion: dextrose 40 g, meat peptone 5 g, casein peptone 5 g and agar 15 g. This was previously sterilized by autoclaving and

transferred to sterile polystyrene Petri boxes of 90 x 15 mm. To obtain conidia, the boxes were incubated for three weeks at a temperature of 25 ±1 °C and a photoperiod of 24 h dark. The extraction of conidia was carried out by scraping the surface and then suspended in 0.03% Tween 80 solution. The quantification of conidia was carried out in a Neubauer chamber, according to the method proposed by Goettel and Douglas (1997).

Table 1. Reference of the fungi used in the evaluation of pathogenicity against *D. opuntiae*.

Species	Key	Host insect	Location
B. bassiana	Bb1	Hymenoptera	Jalisco, Mexico
B. bassiana	GHA	Mycotrol®	USA
M. anisopliae	Ma129	*Tetranychus urticae*	Tecomán, Colima, Mexico
M. anisopliae	Ma130	*T. urticae*	Tecomán, Colima, Mexico
L. lecanii	*ARSEF 2009	*Toxoptera citricida*	Tucumán, Argentina
L. lecanii	*ARSEF 974	Aphids	Venezuela

*= Isolates with the prefix ARSEF, belong to the isolates collection of the Agricultural Research Service of Entomopathogenic Fungi, USA.

Pathogenicity evaluation

To evaluate the pathogenicity of the different strains on the mortality of *D. opuntiae*, the nopal cuts containing second instar nymphs were placed in transparent disposable cups and sprinkled with conidial suspensions of the six isolates. A single dose of 1 x 10^8 mL^{-1} conidia suspended in 0.03% Tween 80 solution was applied, plus a control where 0.03% Tween 80 solution was applied. The sprays were made in a spray tower with the following characteristics: acrylic cylinder 30 cm in diameter and a height of 50 cm, which had an inclination of 45° at 30 cm in height. All treatments were subjected to a pressure of 10 psi. After spraying, the treated experimental units were placed in transparent plastic containers and kept at a temperature of 26 ±1 °C, 60% relative humidity and a photoperiod 12:12 (light:dark). The mortality of the nymphs was evaluated during a period of 192 h with intervals of 24 h after spraying. The extraction and quantification of dead insects was carried out every 24 h and each of them was placed in a humid chamber to accelerate the sporulation of the fungus and thus confirm the infection of the insect (Butt and Goettel, 2000).

Virulence evaluation

According to the results obtained in the pathogenicity test, the three isolates that generated the highest mortality (Ma130, Ma129 and 974) were selected to evaluate their virulence on *D. opuntiae*. For this, nopal cuts containing second instar nymphs were placed in transparent disposable cups and four doses of conidia of the three isolates (10^6, 10^7, 10^8 and 10^9 conidia mL^{-1}) suspended in 0.05% Tween 80 solution were sprayed, plus a control where 0.05% Tween 80 solution was applied. To perform the atomizations, the same procedure was followed as in the previous section. After the spraying, the treated experimental units were placed in transparent plastic containers, which were introduced, in turn, into a breeding chamber with the following conditions: relative humidity (60%), photoperiod (12:12) and a temperature of 26 ±1 °C. The mortality of the nymphs subjected to the three treatments was evaluated for 192 h, at intervals of

24 h after the spraying. Daily extraction and quantification of dead insects was carried out and the indications of Butt and Goettel (2000) were followed. The mortality percentage was calculated according to Abbott's formula (1925).

$$\% \text{ of mortality} = \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100$$

Statistical analysis

Regarding pathogenicity, a completely randomized design was used, with six treatments (isolations) plus one control, three replicates in time and two pseudo-replicates each. In each of the treatments there were two repetitions, thus having 12 units, plus one control. The mortality percentages of nymphs were transformed by arcsen ($\sqrt{x/100}$) prior to the analysis of variance (Castillo, 2007). In the determination of virulence, a completely randomized experimental design was used, with 12 treatments plus one control and was repeated four times in time. Also, a Probit analysis was performed to estimate the CL_{50} and CL_{95} of each isolate with a 95% confidence limit. An analysis of variance and a multiple mean comparison of both the isolates and their concentrations with the Tukey test ($p \leq 0.05$) were also performed using the Statistical Analysis System (SAS Institute, 2012).

Results and discussion

Determination of pathogenicity

The results of the study showed that *M. anisopliae* had the highest mortality of nymphs with 41.3%; subsequently, *L. lecanii* followed, which overall caused a mortality of 34.4%. On the contrary, the species that obtained the lowest mortality was *B. bassiana* with only 19.7% (Figure 1). When the effectiveness by isolation was evaluated individually, the treatment that obtained the highest mortality was *M. anisopliae* with the Ma130 isolate, which registered a mortality of 51.1%, followed by the isolation 974 of *L. lecanii* with 38.8% of mortality. The Ma129 and 2009 treatments belonging to the species *M. anisopliae* and *L. lecanii*, were statistically similar with 31.5 and 29.9%, respectively. On the other hand, the isolates that showed less effectiveness corresponded to *B. bassiana*, where the GHA isolate obtained 22.6% and the lowest effectiveness was recorded by the Bb1 isolate with only 16.5% mortality (Table 2). The experiment recorded a mortality of less than 10% in the control, which makes it acceptable and states that the protocol for inoculation of entomopathogenic fungi was effective with respect to innocuousness.

The analysis of the results of the six strains (GHA, Bb1, Ma129, Ma130, 974 and 2009) applied to a single concentration (1×10^8 conidia mL^{-1}) showed that all isolates have pathogenicity against *D. opuntiae*. The most effective fungi were *M. anisopliae* and *L. lecanii*, in the six isolates tested in the bioassay. The data show that there are statistical differences, even in isolates belonging to the same species, this differentiation can be associated to the virulence characteristics of each isolation, as well as to the influence exerted by the type of host from which its isolation originated.

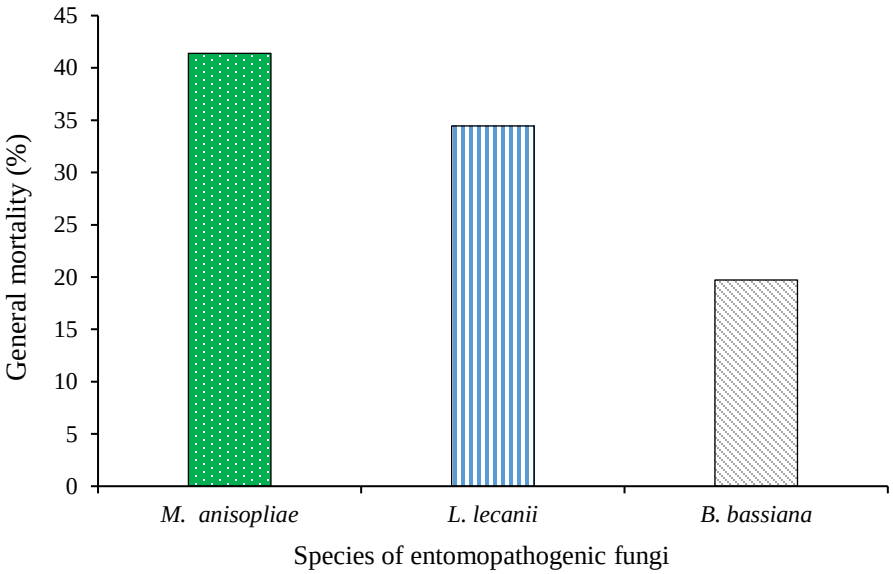

Figure 1. General mortality by species of entomopathogenic fungi on second instar nymphs of *D. opuntiae*.

Table 2. Mortality (%) of second instar nymphs of *Dactylopius opuntiae* treated with six isolates of entomopathogenic fungi.

Species	Strains	Mortality (% ±EE[*])
M. anisopliae	Ma 130	51.12 ±2.04[a]
L. lecanii	974	38.85 ±1.64[b]
M. anisopliae	Ma 129	31.58 ±2.06[c]
L. lecanii	2009	29.98 ±0.85[c]
B. bassiana	GHA	22.69 ±1.33[d**]
B. bassiana	Bb1	16.56 ±1.21[e]
Control	Control	8.81 ±0.7[f]

[*]= different letters indicate differences according to the Tukey test ($p \leq 0.05$). EE= standard error.

The most pathogenic fungus was *M. anisopliae* isolation Ma 130, this behavior can be attributed to the fact that this species has the quality to infect insects and soft body mites such as *Tetranychus urtice* (Mugisho *et al*., 2015), as well as the size of the conidium, regarding the body of the nymph of *D. opuntiae*, it may have more influence on mortality, since the area of contact with the insect is greater, when presenting larger conidia, compared to the species of *B. bassiana* and *L. lecanii* (Humber, 1997).

Actually, few pathogens have been naturally isolated to be used in the control of scales and probably the fungi of greater importance against these insects are those belonging to the genus *Lecanicillium* (Liu *et al*., 2009). In the case of the fungus *L. lecanii*, pathogenicity studies were carried out on insects belonging to the Matsucoccidae family, obtaining 61.3% mortalities in

second instar nymphs at a concentration of 1 x 10^7 conidia mL^{-1} (Liu *et al*., 2014). In the present study, the mortalities obtained with this fungus caused intermediate mortality values between 29.9 and 38.8% for the 2009 and 974 isolates, respectively.

In bioassays performed on *Phoenicococcus marlatti* scales, it was observed that the fungus *B. bassiana* did not manage to parasitize the insect (Asensio *et al*., 2005). Although in this study the registered mortality values were the lowest Bb1 (16.5%) and GHA (22.9%, Santos *et al.* (2011) when evaluating the effect of the fungus *B. bassiana* mixed with protective agents on nymphs of first instar of *D. opuntiae*, detected that by including a sunscreen (Oxybenzone®) in the formulation of the fungus, mortality values of 46.5% were reached, which is higher than those recorded in the present study, where *B. bassiana* (GHA) obtained a maximum mortality of 22.6%.

This difference can be associated to the fact that the sunscreen can accentuate its effect on mortality, when combined with the fungus. It is worth considering that *D. opuntiae* naturally has a high natural mortality rate in first instar nymphs since they do not yet develop their protective cover, due to this, the higher mortality values obtained by Santos *et al.* (2011) may be related to the bioassays performed with nymphs of first instar, while in this investigation they were applied in older nymphs, which present a greater quantity of waxy filaments, which could interfere in the contact of the fungus with the body of the insect. In this regard, Vigueras *et al*. (2009) highlight that when degrading the waxy cover mortality can reach up to 35% in nymphs of first instar.

One of the great advantages of using entomopathogens is that they can be combined with some plant extracts in insect management strategies. Da Silva *et al*. (2016) observed that when *Fusarium incartanum-equiseti* and aqueous extracts of *Ricinus communis* are applied, they can cause up to 100% mortality in *D. opuntiae*, although bioassays were also applied to nymphs and not adults. In studies with other scales, Jin-Hua *et al*. (2014) isolated, identified and evaluated the pathogenicity of the fungus *Fusarium incarnatum-equiseti* that naturally infects the soft scale *Coccus hesperidum* recording the first report of the genus *Fusarium* on this insect, this infers that this fungus could present pathogenicity on other scale insects, although this species was not evaluated in this study.

This potential was recently verified with excellent results in laboratory studies, in combination with plant extracts, for the control of *D. opuntiae* in Brazil (Da Silva *et al*., 2016). When testing *M. anisopliae* and *B. bassiana*, it was observed that the latter recorded the best results on nymphs of *D. opuntiae*, also emphasizing that the combination of both species could improve their response. This trend could not be confirmed in this investigation. A dose similar to that used in this study was also used with *B. bassiana* for the control of *Matamasius spinoleae*, another important *Opuntia* pest in Mexico, obtaining mortality values of 85% (Tafoya *et al*., 2004).

Determination of virulence

To determine the virulence of second instar nymphs of *D. opuntiae* under laboratory conditions, three isolates of entomopathogenic fungi from two species (*M. anisopliae* and *L. lecanii*) were evaluated, which recorded the highest values in pathogenicity. The virulence of each isolate

was determined by estimating the CL_{50} and CL_{95}, expressed in conidia mL^{-1}, based on the mortality obtained on the eighth day after inoculation of the fungi. The isolations tested caused mortality in *D. opuntiae*, being this dependent on the concentration (Figure 2). It was found that the Ma130 isolate (*M. anisopliae*) showed the highest effectiveness, followed by the 974 isolate (*L. lecanii*) and finally the Ma129 isolate, with the lowest effectiveness of the three isolates. Likewise, a positive relationship was observed between the concentration of the applied isolates and mortality, in such a way that as the concentration increases there is an increase in mortality.

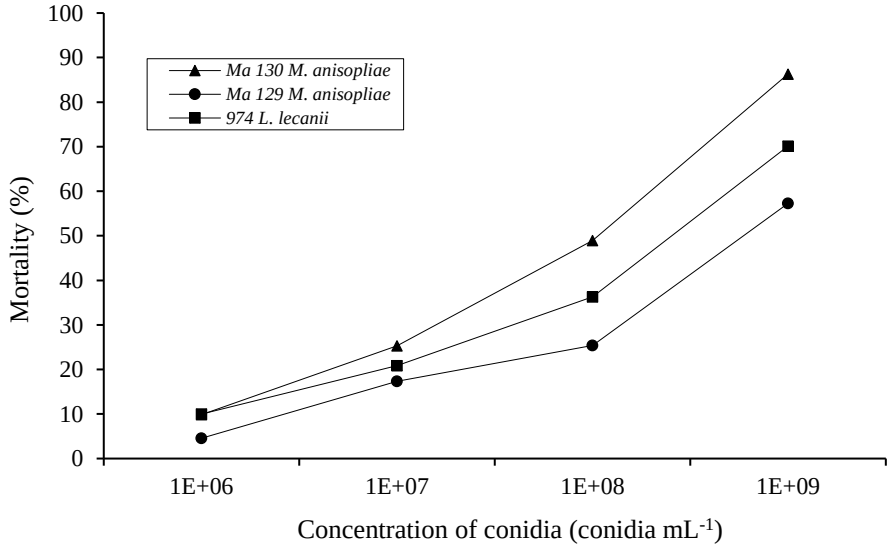

Figure 2. Corrected mortality (%) of *D. opuntiae* at different levels of concentration of conidia in three isolates evaluated.

Table 3 shows the average mortality recorded and the mortality corrected according to Abbott's formula (1925), which highlights that the Ma130 isolate of the species *M. anisopliae* showed greater effectiveness in the four concentrations tested, the values Average mortality ranged between 17.5% (1 x 10^6 conidia mL^{-1}) and 87.5% (1 x 10^9 conidia mL^{-1}). The three highest concentrations showed significant differences, among them; however, the lowest concentration (1 x 10^6 conidia mL^{-1}) showed an average mortality of 17.5%, which was not statistically different, with respect to the control (8.3%).

L. lecanii 974 recorded the best effectiveness, after isolation Ma 30, since it obtained values of 72.5% at a concentration of 1 x 10^9 conidia mL^{-1}. In contrast, the lowest mortality was observed in the treatment with the concentration of 1 x 10^6 conidia mL^{-1}, where only 17.5% was recorded, which does not differ statistically from the control. Regarding the isolation Ma129 of *M. anisopliae*, it had the lowest mortality value, with respect to the other two isolates evaluated. The highest average mortality (60.83%) was obtained with the highest concentration (1 x 10^9 conidia mL^{-1}) in contrast, the lowest average mortality value that this isolation presented (12.5%) was observed with the concentration of 1 x 10^6 conidia mL^{-1} (Figure 3).

Table 3. Observed mortality (% ±EE*) and corrected using the Abbott formula.

Isolation concentrations	Observed mortality (%)	Corrected mortality (%)
Ma 130- 10^9	87.5 ±4.59[a]	86.36
Ma 130- 10^8	53.33 ±4.91[b]	49.09
Ma 130- 10^7	31.67 ±3.47[c]	25.46
Ma 130- 10^6	17.5 ±3.7[cd]	10
Ma 129- 10^9	60.83 ±4.98[a]	57.27
Ma 129- 10^8	31.67 ±3.47[b]	25.46
Ma 129- 10^7	24.17 ±4.38[bc]	17.28
Ma 129- 10^6	12.5 ±3.94[c]	4.55
974- 10^9	72.5 ±4.98[a]	70
974- 10^8	41.67 ±2.15[b]	36.37
974- 10^7	27.5 ±2.85[c]	20.91
974- 10^6	17.5 ±2.85[cd]	10
Control	8.33 ±0.96	-

*= different letters, between concentrations of isolates indicate differences according to the Tukey test ($p \leq 0.05$). EE= standard error.

In the Table 4 shows the CL_{50} and CL_{95} values (including the confidence limits) and the regression equations obtained from the Probit analysis. The mean lethal concentration (CL_{50}) estimated for the isolation Ma130 (*M. anisopliae*) resulted in 6.63 x 10^7 conidia mL^{-1}, in the case of isolation Ma129 the CL_{50} obtained a value of 6.56 x 10^8 conidia mL^{-1} and for the isolation 974 of the species *L. lecanii* the estimated value of the CL_{50} obtained was 2.07 x 10^8 conidia mL^{-1}. Based on this information, it is possible to consider that the most effective isolation is Ma 130, which shows a lower requirement in the concentration to kill 50% of the population under study in a given period of time. In order of decreasing effectiveness, the species *L. Lecanii* (974) followed and finally the treatment with less effectiveness turned out to be the Ma129 isolate.

Table 4. CL_{50} and CL_{95} of three isolates of entomopathogenic fungi on second instar nymphs of *D. opuntiae*.

Isolation	CL_{50} (95% LC)	CL_{95} (95% LC)	Probit regression equation
Ma 130	$6.63x10^7$ ($4.83x10^7$-$1.03x10^8$)	$8.45x10^9$ ($3.47x10^9$- $2.92x10^{10}$)	Y= -6.1119+0.7814X
Ma 129	$6.56x10^8$ ($3.36x10^8$-$1.67x10^9$)	$4.01x10^{11}$($7.04x10^{10}$-$6.60x10^{12}$)	Y= -5.2041+ 0.5902X
974	$2.07x10^8$($1.19x10^8$- $4.12x10^8$)	$1.10x10^{11}$ ($2.59x10^{10}$- $1.01x10^{12}$)	Y= -5.0167 + 0.6032X

On the other hand, Pereira *et al*. (2011) observed that the DL_{50} of *M. anisopliae* var. *anisopliae* recorded a value of 7.3 x 10^6 conidia mL^{-1} against nymphs of second instar of *D. opuntiae*. The foregoing, may be related virulence characteristics of the isolation, such as the production of enzymes (proteases, chitinases and lipases) which facilitate the penetration of the cuticle of the insect and allow to reach inside it (hemocele) (Schrank and Vainstein, 2010). Although the use of *M. anisopliae* against scale insects has been little used, because this fungus is naturally associated with members of other orders such as: Coleoptera, Isoptera or Orthoptera (Shahid *et al*., 2012), has demonstrated the effectiveness of this fungus against mealybugs (*Pseudococcus viburni*).

The concentration of conidia had a positive linear response with mortality, given that at a higher concentration the mortality increased. This can be related to the amount of viable spores that manage to position themselves on the cuticle of the insect and that later will germinate, in this way they can have greater opportunity to infect the insect by the amount of spores present (Table 5).

Table 5. Estimation of conidia for each treatment applied.

Treatment	Conidia average
Ma 130- 9	66.67
Ma 130- 8	25
Ma 130- 7	11.33
Ma 130- 6	3.67
Ma 129- 9	42.33
Ma 129- 8	19
Ma 129- 7	4
Ma 129- 6	1.33
974- 9	54
974- 8	44.33
974- 7	17.67
974- 6	11.67

An important aspect to highlight is that the mortality of the nymphs of *D. opuntiae* started from the first day after the application of the fungi. Between the third and sixth day, the highest mortality rate was observed in the three isolates and it shows a tendency to stabilize after the eighth day. The Ma130 isolate showed a greater effectiveness of accumulated mortality with respect to time, by eliminating a greater number of nymphs in less time, in relation to the other two isolates evaluated (Figure 3).

The three isolates of entomopathogenic fungi evaluated, two belonging to the species *M. anisopliae* and one of the species *L. lecanii*, although they show different degree of virulence, have the potential to cause infection in second instar nymphs of *D. opuntiae*. The results of the study showed that Ma130 isolation of *M. anisopliae* was most effective. This species is the most common entomopathogenic fungus around the world, given its natural availability in the soil and also because of the high number of host insect species (Senthil-Nathan, 2015). In the investigation, this isolation had a greater effectiveness in percentage of mortality, as well as the lowest CL_{50} (6.63 x 10^7 conidia mL^{-1}), in comparison with the other two isolates evaluated.

In this study, the Ma129 isolate belonging to the species *M. anisopliae* was also evaluated, which showed a lower effectiveness compared to the other two isolates. The two isolates of *M. anisopliae* evaluated in this investigation recorded their maximum mortality after the sixth day after spraying. A similar trend, observed Oreste *et al*. (2016) when studying the effect of an isolation of *M. anisopliae* on whitefly, registering mortality values of 94.1%, on the seventh day after the application of fungi.

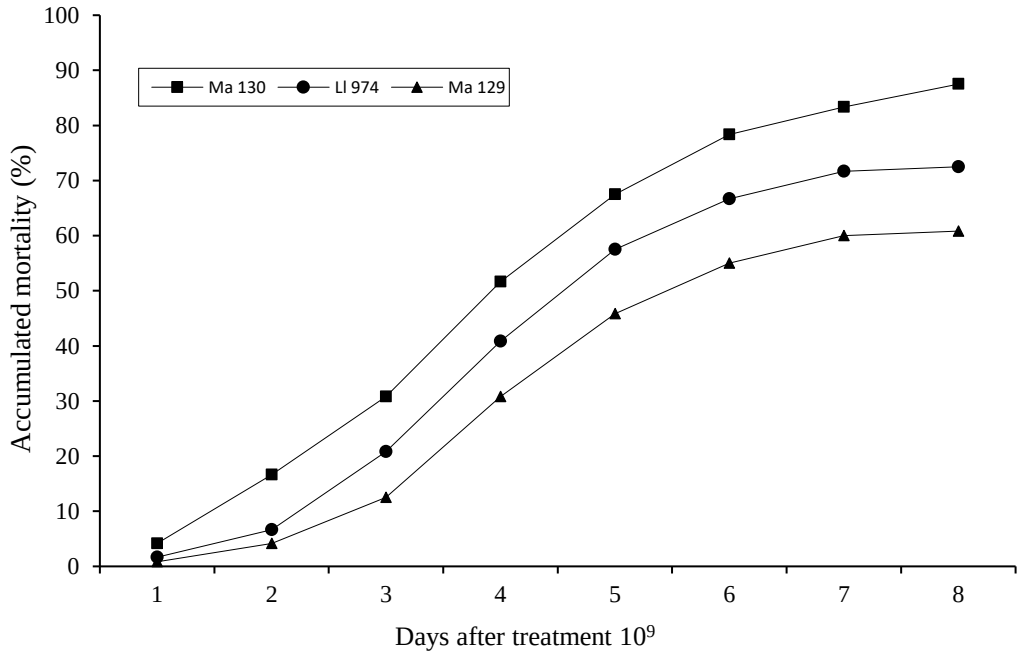

Figure 3. Accumulated mortality (%) of three isolates of entomopathogenic fungi in second instar nymphs of *D. opuntiae*, under laboratory conditions.

Regarding the isolation 974 belonging to the species *L. lecanii*, it presented an interesting effectiveness in the evaluation of virulence, with respect to the other two isolates evaluated, since it obtained a value of the CL_{50} of 2.07 x 10^8 conidia mL^{-1}. This species also has a high number of host insects; however, it is the main pathogen of hemiptera such as scales, aphids and whitefly (Sujeetha and Sahayaraj, 2014). For example, Telli *et al*. (2014) evaluated the mortality of the scale *Coccus hesperidium* L. by inoculating the fungus *L. lecanii* in laboratory conditions and obtained an average of 47.5% mortality at a concentration of 1 x 10^7 conidia mL^{-1}. The authors conclude that this fungus has the potential to be used in integrated management plans of phytophagous insects belonging to the order Hemiptera.

On the other hand, Liu *et al*. (2014) analyzed the virulence of two isolates of the fungus *L. lecanii* against second instar nymphs of the *Matsucoccus matsumurae* scale and obtained mortality values that ranged between 53.6 and 61.3% for isolates V34504 and V34505, respectively. Higher values of mortality were presented after the sixth day of inoculation, which coincides with that observed in this investigation. Likewise, Xie *et al*. (2010) concluded that this fungus invades scale insects and found that conidia damage the cuticle and penetrate the integument through a combination of mechanical forces and degrading enzymes.

According to the results obtained in this research it is emphasized that the control of *D. opuntiae* by entomopathogenic fungi can be a viable alternative, taking as reference the mortality obtained in laboratory conditions. However, it is necessary to consider that the effectiveness of entomopathogens under field conditions is lower than that found in laboratory conditions; however, its effectiveness in the field can be increased by the use of good management practices in each of

the stages of the cultivation of fungi, among which can be considered to ensure the quality of the product to be applied, use of correct doses, methods and time of application, and monitoring, among other factors.

Conclusions

The six isolates evaluated showed a differential behavior even being of the same species, with respect to pathogenicity against *D. opuntiae*, at a concentration of 1 x 10^8 conidia mL^{-1}. *M. anisopliae* (Ma130) recorded the highest mortality under laboratory conditions.

Consistently, the Ma130 isolate resulted with the highest virulence against second instar nymphs of *D. opuntiae*. The value of the CL_{50} obtained is 6.63 x 10^7 conidia mL^{-1}. The highest mortality rate of nymphs of second instar *D. opuntiae* occurred between 3 and 6 days after the application was made.

Isolation Ma130, belonging to the species *M. anisopliae*, could be included in the strategy of integrated management of *D. opuntiae* as an alternative to the use of agrochemicals.

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