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In vitro evaluation of native entomopathogenic fungi and neem (*Azadiractha indica*) extracts on *Spodoptera frugiperda*

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Abstract. The control of Spodoptera frugiperda is based on synthetic insecticides, so some alternatives are the use of entomopathogenic fungi (EF) and neem extract. The objective of the study was to evaluate in vitro effectiveness of native EF and neem extracts on *S. frugiperda* larvae. Six EF were identified by DNA sequencing of ITS regions from three EF (*Fusarium solani, Metarrhizium robertsii, Nigrospora spherica* and *Penicillium citrinum*). They were evaluated in concentrations of 1×10^8 spores/mL. In addition, a second bioassay was carried out evaluating only *F. solani*, *M. robertsii* and *N. sphaerica* and the addition of vegetable oil. On the other hand, extraction of secondary metabolites from neem seed (*Azadirachta indica*) was carried out by performing, mass (g) and solvent volume (mL ethanol and water) combinations, which were subjected to microwaves and ultrasound. Subsequently, these extracts were evaluated in concentrations of 3%, 4% and 5%. A survival analysis was performed for each of the bioassays. With respect to the results of the first bioassay, *F. solani* obtained a probability of survival of 0.476 on the seventh day, while in the second bioassay, *M. robertsii* obtained 0.488 survival probability. This suggests that the expected percentage of larvae that stay alive on the sixth day is 48.8%. However, in the evaluation of the neem extract the combination 1:12/70% to 4% caused 84% mortality of larvae. The use of native HE and neem extracts has potential for the control of *S. frugiperda*.

Keywords: Bioinsecticide; Biological control; Fall armyworm; Extracts; Seed.

INTRODUCTION

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) commonly called the armyworm is an important pest in many crops such as sorghum, rice, cotton and alfalfa (Nexticapan-Garcéz et al., 2009). However, it shows greater preference for maize and also during almost all its life cycle can cause losses greater than 30% (Reséndiz et al., 2017). For the control of *S. frugiperda*, mainly synthetic insecticides are used, which can cause adverse effects on the environment and human health (García-Gutiérrez et al., 2012). Therefore, alternatives that minimize the aforementioned adverse factors are sought, one of which is the use of entomopathogenic fungi (EF) (García et al., 2011); these can be specific for certain groups of insect pests, that is, they only affect certain individuals (Rios et al., 2017); besides that they can be produced and applied with conventional equipment in their place of origin (González-Maldonado et al., 2015). Around 750 species of EF have been reported affecting pests of economic importance, among them some genres as, *Metarhizium*, *Beauveria*, *Isaria*, *Verticillium* and *Fusarium* isolates (Motta-Delgado and Murcia-Ordoñez, 2011). For its use, it is necessary the correctly identify EF, since traditionally, it is done by morphological characters (Allende et al., 2013). However, it is only possible to determine the genus and sometimes it is difficult to differentiate among them (Rivera et al., 2016). The identification by morphological characters can be complemented with other

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techniques; among them, molecular identification, an efficient tool that involves DNA extraction, amplification and sequencing processes (Berlanga-Padilla et al., 2016).

Neem, *Azadirachta indica* (A. Juss) (Meliaceae: Sapindales), is also used for lepidoptera pests control such as *S. frugiperda* (García-Gutiérrez et al., 2012), because, it possesses bioactive compounds with insecticide activity (Reyes and Fernández, 2013). Within these compounds are mentioned phenols, quinones and flavonoids (Muñiz-Reyes et al., 2016), with azadirachtin being the main active ingredient (Reyes and Fernández, 2013). This compound regulates growth, causes repellency, decreased oviposition and cessation of feeding in insect pests such as *S. frugiperda* and *S. eridania* (Constanski et al., 2016). However, the effectiveness of any type of pest control depends on multiple interactions, where the crop, pest and environment are involved (Do Prado et al., 2014). Therefore, it is convenient to carry out evaluations under controlled environments that allow knowing and predicting the effectiveness of a pest control strategy; strategies that must subsequently be evaluated and validated in field conditions or uncontrolled environments (Silva et al., 2002). Therefore, the objective of this study was to evaluate *in vitro* effectiveness of native entomopathogenic fungi and neem extract on *S. frugiperda* larvae.

MATERIALS AND METHODS

Site of sampling and establishment of the experiment

In different agricultural areas of the Güemez county, Tamaulipas, Mexico, larvae of *S. frugiperda* with symptoms of EF infection were collected. Additionally, 1 kg of agricultural land was collected from this county and from Abasolo county, Tamaulipas. The bioassays were carried out in the Microbiology laboratory of the Integral Laboratory Center, Faculty of Engineering and Sciences-the Autonomous University of Tamaulipas.

Isolation of entomopathogenic fungi

In this step, 20 grams of soil were placed on Petri dish with two larvae of *Tenebrio* sp. which were used as a trap insect to obtain EF. The larvae were checked every 24 hours until the presence of EF was observed, then they were disinfected with 3% NaClO and washed three times with sterile distilled water and allowed to drain on paper towels under a laminar flow hood. Once disinfected, larvae were placed on papa dextrose agar culture medium and incubated at 25 ± 2 °C for 6 days. Subsequently, from the micellar growth developed into the larva, EF isolation was performed until the isolates were pure. The disinfection and purification procedureswere repeated with the fungal isolates collected from *S. frugiperda*. From both procedures, those EF that showed different macroscopic characteristics were selected, and were purified by hyphae tip and planted on papa dextrose agar culture medium.

Morphological identification entomopathogenic fungi

The morphological identification of the EF was done using the taxonomic Barnett and Hunter (1998) keys. To perform this step, assemblies were made from mycelium of fungi stained with lactoglycerol blue in slide and coverslips to observe their microscopic characteristics, such as shape, size of hyphae and spores, under an optical microscope in 400X magnifications. Six EF were obtained, four of which were isolated from the Güemez soil (P1, P2, P3 and P4), one obtained from *S. frugiperda* larvae (SFP2), this insect was collected from the same municipality and another one from Abasolo soil (A1).

Molecular identification entomopathogenic fungi

Isolation of genomic DNA from six EF was carried out following the methodology described by Barth and Gallardin (1996), in this case, 2 gram of mycelium of each isolated fungus were employed. Then, the polymerase chain reaction (PCR) was performed to amplify the ITS region using the primer ITSR 5´-GGAAGTAAAAGTCGTAACAAGG-´3 and ITSF 5´- TCCTCCGCTTATTGATATGC-´3. The reaction mixture was prepared with 14.5 µL of water mili-Q estéril, 3.5 µL Buffer 10x, (1.0 mL), 2.0 µL of each primer (10 µmoles/UI), 0.5 µL de dNTP´s (10mM), 0.5 µL (5 U/µL) of Taq ADN polimerasaand 2 µL of ADN, obtaining a total volume of 25 µL. Subsequently, samples were placed in a thermal cycler (AxygenMaxygene) with the following conditions: an initial step of denaturation to 94 °C for 10 minutes, followed by 35 cycles at 94 °C for 1 minute for P1, P2, P3, P4, A1 and SFP2 DNA denaturing,subsequently, annealing for 1 minute at 50.3 °C for P4, SFP2 and P3 DNA, besides, a polymerization step at 54 °C for 1 minute, using P1, P2 and A1 DNA, after that, an extension step for 1 minute at 72 °C. Finally, a period of one cycle of final extension of 10 minutes to 72 °C for P4, SFP2 and P3 DNA, in contrast for, P1, P2 and A1 DNAby 5 minutes to 72 °C, in all cases, samples were maintained at 4 °C.Then, PCR products were visualized on a 1.5% agarose gel using 3 µL de ADN of reaction, 2 µL of ethidium bromide, 2 µL of bromophenol blueand 1 µL of molecular marker, bands were visualized using an ultraviolet transilluminator (SPECTROLINE). Subsequently, they were purified to be sequenced in both chains. The sequences obtained were compared by homology with data from the National Center for Biotechnology Information (NCBI) using the GenBank database.

Extraction of secondary metabolites from neem seeds

Neem mature seeds were collected in the Victoria county, Tamaulipas. Subsequently, they were dried in an oven at 65 °C for 72 hours and ground in a manual mill. Two combinations were madefrom the ground seeds, (mass of seed) and volume (mL of solvent), the latter in 70% ethanol and 0% distilled water, which corresponded to: 1:12/70% and 1:12/0%. Subsequently, samples for extraction of secondary metabolites were submitted to ultrasound (Bransson 5500) with a volume of 5 L at 40 kHz, for 20 minutes and microwave (Mars 6 CEM) with carousel of 24 reactors of 50 mL with double cap,

this process was carried out for 5 minutes at 70 °C with a power of 800 W.

In vitro evaluation of entomopathogenic fungi

Two evaluations were carried out, in the first, seven treatments were they used, the six EF and a control without inoculum (sterile distilled water). In this case, 30 seconds instar larvae were used in each treatment, which were immersed for 3 seconds in a suspension 1×10^8 spores/mL of the treatment to be evaluated, it is worth mentioning that the spore count was performed by Neubauer camera and by observation in a microscope, the larvae were placed individually in plastic cups, due to their cannibalistic habit and were fed with tender pieces of corn leaf. The number of individuals killed in each treatment was recorded every 24 hours for 6 days.

In the second bioassay, P1, P3 and SFP2 were evaluated, because in the first bioassay they showed greater effectiveness and as a control was used sterile distilled water. Fifty second stage larvae were used for each of the EF, which were previously weighed before being treated. The treatmentapplication was carried out as the first bioassay with modifications, since unlike the first bioassay, a 15% sunflower vegetable oil was used as adherent of the application. Likewise, the larvae were kept under the same conditions as the first bioassay for 10 days and every 24 hours the number of dead individuals was recorded.

In vitro evaluation of neem extract

The extract 1:12/70% (A) and 1:12/0% (B) combinations were evaluated at concentrations of 3%, 4% and 5% and distilled water was used as a sterile control. In this step, 30 seconds stage larvae were used in each of the seven treatments; the extract application was made using an atomizer, a total of 5 mL for each of the extracts in their respective concentrations. Then, the larvae were placed individually in plastic cups to avoid cannibalism and were fed with small tender pieces of corn leaves. They were evaluated for 6 days and every 24 hours, the number of dead individuals in each treatment was recorded.

Statistic analysis

For each bioassay, a survival analysis was performed where nonparametric method using the Kaplan-Meier estimator (Klein and Moeschberger, 2005) was performed, the survival library (Therneau, 2018) and the R program were used (R Core, 2016) for these analyses.

RESULTS

Morphological and molecular identification of entomopathogenic fungi

Six EF were identified morphologically, three (A1, P3 and P4) of them were classified taxonomically within the *Fusarium* genus, which presented macro and microconidia septate, the cottony white mycelium which became purple (Fig. 1). The molecular identification determined that A1, P3 and P4 belonged to *Fusarium solani*. While, EF P1 corresponded taxonomically to *Metarrhizium* genus, which presents conidia in chain, with a white color at the beginning of its growth and later it turns to olive green. The molecular identification of this EF determined that it is belonged to *M. robertsii*. SFP2 corresponded taxonomically to the *Nigrospora* genus, and molecularly to *N. sphaerica*, this isolate is distinguished by conidiophores and chain conidia, in which chain conidia occured in branched form (Fig. 2). On the other hand, EF P2 was identified taxonomically as *Penicillium* genus and molecularly as *P. citrinum* (Table 1).

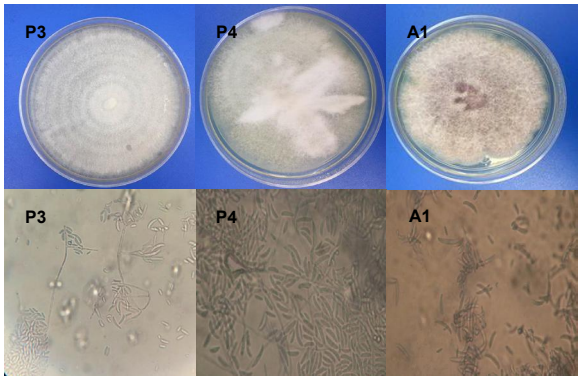

Fig. 1. Macroscopic and microscopic characteristics of *Fusarium* sp. (P3, P4 and A1).

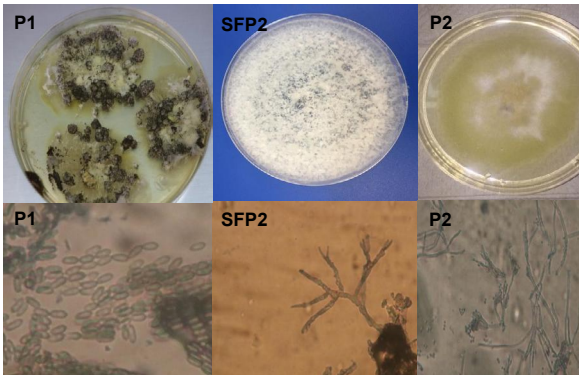

Fig. 2. Macroscopic and microscopic characteristics of *Metarhizium* sp. (P1) *Nigrospora* sp. (SFP2) and *Penicillium* sp. (P2).

Evaluation of entomopathogenic fungi on *S. frugiperda*

In the first evaluation, three of the fungal isolates stand out, *F. solani* (P3) in which the larvae recorded a survival probability of 0.476 at day seven, that is, 47.6% of the *S. frugiperda* larvae are still alive on that day. On the other hand, the EF *N. sphaerica* obtained a survival probability of 0.567, for which 56.7% of the larvae survive to day eight. In particular, larvae treated with *M. robertsii* they got a probability of survival from 0.575 to day seven. That is, it is expected that after seven days of application 57.5% of larvae will survive (Fig. 3). In this case, *F. solanni* (P3) was highlighted for causing 52.4% of *S. frugiperda* larvae mortality.

Table 1. Molecular identification of entomopathogenic fungi obtained from the Abasolo and Güémez counties, Tamaulipas, México.

Isolation code	Fungi species	Identity (%)	Location	Número de accesión
A1	*Fusarium solani*	100	Abasolo	KY814675.1
P3	*Fusarium solani*	98	Güémez	KX688605.1
P4	*Fusarium solani*	100	Güémez	MF663680.1
P1	*Metarhizium robertsii*	100	Güémez	MF681620.1
P2	*Penicillium citrinum*	100	Güémez	KX817797.1
SFP2	*Nigrospora sphaerica*	100	Güémez	KX985964.1

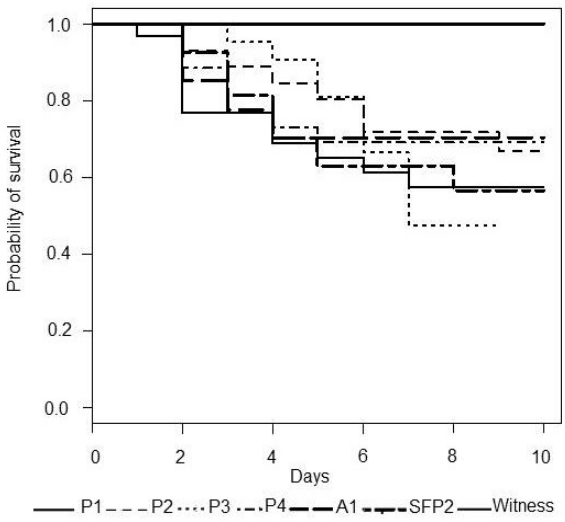

Fig. 3. Survival function of *S. frugiperda* larvae treated with entomopathogenic fungi. ***M. robertsii*** (P1), ***P. citrinum*** (P2), ***F. solani*** (P3), ***F. solani*** (P4), ***F. solani*** (A1) y ***N. sphaerica*** (SFP2).

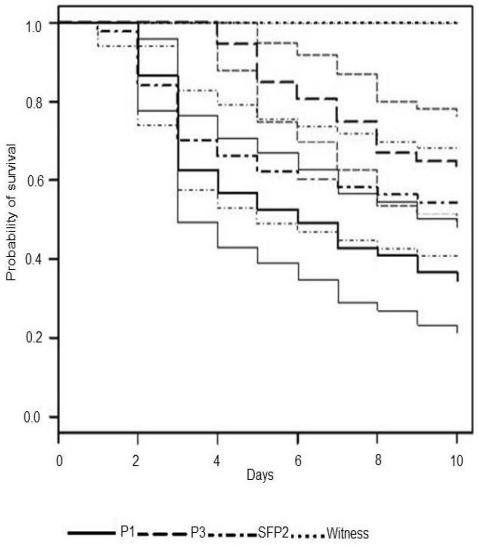

Fig. 4. Survival function and confidence intervals at 95% of *S. frugiperda* larvae treated with three entomopathogenic fungi ***M. robertsii*** (P1), ***N. sphaerica*** (SFP2) and ***F. solani*** (P3) and with addition of vegetal oil.

The effect of EF on *S. frugiperda* larvae survival was not constant over time (Fig. 4). From day two and until day seven, after application, only significant differences ($p = 0.05$) for *S. frugiperda* larvae survival was recorded between the EF P1 and P3 isolates, in this period, the EF presented less survival with respect to P3; however, from day seven to day nine after application, there was no significant differences ($p = 0.05$) for *S. frugiperda* larvae survival in the EF evaluated. On the other hand, on day nine, again significant differences ($p = 0.05$) were recorded between the EF P1 and P3 fungal isolates, the EF P1 recorded lower *S. frugiperda* larval survival with respect to the EF P3 isolate.

Evaluation of neem extract on *S. frugiperda* larvae

The extract of the combination 1:12/70% treatment at a concentration of 3% on the second day was observed 0.433 survival probability, which is why 43.3% of the larvae are expected to survive. In the concentration at 4% the probability was 0.266 on day two, that is, 26.6% of larvae they were alive; particularly in this combination all the larvae at day four died. On the other hand, using the concentration of 5% a probability of 0.500 was observed, that is, 50% of larvae can survive on day two.

On the other hand, with the treatment 1:12/0% to 3% on the sixth day, 0.400 post-application survival was obtained, so it is expected that 40% of the larvae will survive; in the 4% concentration at day five, it is expected that 50% of the larvae

will survive. While in the 5% concentration on the fifth day it is expected that 46.7% of the larvae will survive (Fig. 5).

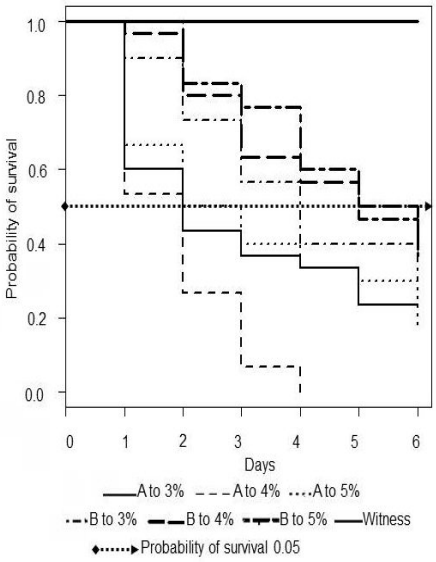

Fig. 5. Survival function of ***S. frugiperda*** larvae treated with two extracts (A) 1:12/70% B) 1:12/0%), at 3 concentrations.

DISCUSSION

Morphological and molecular identification of entomopathogenic fungi

Six EF of soil and larvae of *S. frugiperda* were isolated from the maize crop, of which *M. robertsii and F. solani* excelled due to the number of dead larvae they registered in comparison to the other treatments. It is worth mentioning that the latter is not common to find it as an entomopathogen because it is considered as a cause of diseases in some crops of economic importance. However, Motta-Delgado and Murcia-Ordoñez (2011), considered *Fusarium* spp., as one of the EF of importance for control of agricultural pests. With regard to the latter, some authors such as Sharma and Marques (2018) mention that *F. solani*, besides being a phytopathogen, has been found as an insect pathogen and that it is sometimes in the soil but does not infect plants; this fungus has been found in insects like *Galleria mellonella* Linnaeus (Pyralidae: Lepidoptera). Borges et al. (2010), they found that some secondary metabolites produced by *Fusarium* spp., are used for the control of *Choristoneura fumiferana* (Lepidoptera: Tortricidae) larvae.

On the other hand, *M. anisopliae*, *B. bassiana* and Isaria fumosorosea have been found in soils of corn and sorghum crops, as reported by García et al. (2011). In comparison to the evaluation carried out in this investigation, only *M. robertsii* was found. It is important to note that Lezama et al. (2005) report to *Metarhizium* sp. for the control of larvae of *S. frugiperda*. Regarding *M. robertsii*, Ramanpreet and Michael (2012) not only considered it as an insect pathogen but also as a promoter of root growth in the plant.

EF isolated from soilwere using *Tenebrio molitor* larvae as a trap insect to identify EF native from the Tamaulipas state; whereas in a work performed by García et al. (2011) in the Michoacán state were used *Galleria mellonella*. Therefore, it is suggested that there is a wide diversity of EF species and that it is a function of the origin of the isolates. It should be mentioned that the origin of the isolates and the conditions of the place of origin can determine the presence of EF. According to the above, Nava-Pérez et al. (2012), reported that some insect pathogens have limited range of hosts, while other species of fungi tend to expand it.

On the other hand, the EF of *S. frugiperda* larvae collected in the field with symptoms corresponded to *N. sphaerica*. This fungus is uncommon, however, Amin (2013), evaluated filtrates of this fungus for control of *Meloidogyne*s juveniles (J2). So that, even though, they are of limited presence, they can become infected with pests. The same happens with *Penicillium* sp. however, Burgos-Dueñas et al. (2014) found that *Penicillium* sp. attacks *Pseudips mexicanus* Hopkings, 1915 (Curculionidae: Scolytinae), this insect is considered a pest of pine and oak wood. In addition, they also reported that it is used for *Culex* (L. 1762) (Diptera: Culicidae), control, an insect vector of human pathogens.

Effects of entomopathogenic fungi on *S. frugiperda*

From the six EF evaluated in the first bioassay, the one that obtained the highest mortality on larvae of *S. frugiperda* was *F. solani* (P3). However, the remarkable pathogenicity of *M. robertsii*, which caused 43% mortality with concentrations of 1×10^8 spores/mL on the sixth day is also worth to mention. Angel-Ríos et al. (2015), evaluated *Metarhizium* sp. on larvae of *S. frugiperda* of third stage and caused 55% mortality. The above coincides with that reported by Trujillo et al. (2003) who found that *M. anisopliae* strains for control of *Thrips palmi*, were effective in concentrations of 1×10^8 spores/mL, obtaining an effectiveness of 67.5%. In addition, Lezama et al. (2005) reported *Metarhizium* sp. for control of *S. frugiperda* larvae. On the other hand, the origin of EF determine the degree of virulence, as reported García et al. (2011) who evaluated the germination of native fungal spores and determined that it depends on the host insect; this is due to integument variation, penetration capacity of the EF through the cuticle and that often this penetration may or may not provide the point of support during the infection processes.

In the second bioassay, with addition of vegetable oil, *M. robertsii* on the ninth day caused 52% mortality in the second stage *S. frugiperda* larvae. It should be noted that *M. robertsii* was not isolated directly from the insects. It can be argued that, use of this oil can increase effectiveness of EF spores during the process of infection on the insect. In respect, Cortez-Madrigal et al. (2006), mention that sunflower oil applied to 15% with *Lecanicillium lecanii* (Zimm.) Zare was efficient for *Toxoptera aurantii* Boyer control, it is also mentioned that this oil is considered as an adjuvant that has had positive results in formulation with EF; it is also important to mention that the percentages of oil to be used, as well as some conditions that allow the fungus to be repelled, will depend on its effectiveness.

In this regard, Morales et al. (2014) reported that use of vegetable oils does not affected conidia effectiveness, on the contrary, it maintains the spore for longer and offers greater effectiveness in terms of mortality, but in a longer period. Motta-Delgado and Murcia-Ordoñez (2011) reported that vegetable oil confers the fungus lower humidity availability, since it is an important factor for reproduction effectiveness and host infection.

Evaluation of neem extract on *S. frugiperda* larvae

In the evaluation of the neem seed extract, it was observed that use of ethanol as a solvent, favored the extraction process. Trujillo et al. (2008) reported that use of solvents such as alcohol helps for appropriate extraction of secondary metabolites. Therefore, it is considered that type and amount of solvent used during extraction of secondary metabolites significantly influences solvent effectiveness; since it makes them more available and soluble. In addition, Trujillo et al. (2008) showed that the lower the larval status, the greater the effectiveness of the neem concentration, so in this bioassay the selected larvae were second stage and also, because the secondary metabolites present in the neem and which in turn affect the neuroendocrine system of the insect prevents the process of moulting and this increases the morbidity of the secondary metabolites.

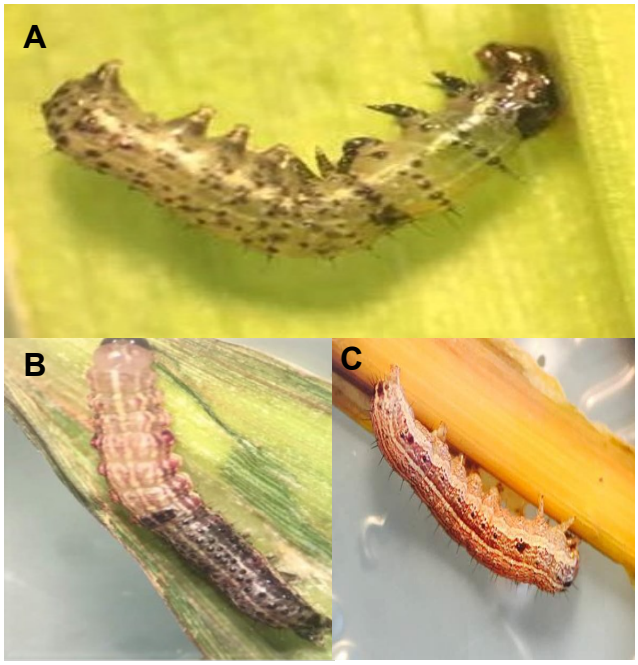

Fig. 6. Effects caused by the neem extracts on armyworm larvae, A) Inhibition of feeding, B) Inhibition of moulting, C) Healthy larva.

Therefore, it was observed in the evaluation that extract A at 4% caused greater mortality compared to extract B in its different concentrations. Besides, in the extract A at 3 and 4%, the larvae mortality occurred by contact, since, it was registered on the first day of post-application evaluation. Using extract B, the mortality of *S. frugiperda* was 52%, so that most larval larval deaths were recorded in the last days of evaluation. As reported by Molina (2001) the ingestion mortality occured around four days. The effects caused by the neem extract in this bioassay on *S. frugiperda* were deformations and decreased feeding (Fig. 6). It is worth of mentioning that, all the treatments caused more than 50% mortality of *S. frugiperda* larvae during the evaluation time, regardless of their concentration. Trujillo et al. (2008) evaluated concentrations of ethanolic extracts from cell suspensions of azadirachtin and found that applying a dose of 5,000 ppm caused the death of 70% of the larval population of *S. frugiperda* and a dose of 10,000 ppm caused the death of the 100% of the noctuidae. In comparison, in this evaluation of two extracts with water and ethanol as solvents; where extract A with 1:12/70% caused 84% mortality of second stage larvae.

CONCLUSION

From the entomopathogenic fungi evaluated, the *F. solani* and *M. robertsii* species showed *in vitro* effectiveness for control of *S. frugiperda* larvae. In addition, use of vegetable oil potentiates the effectiveness of entomopathogenic fungi. On the other hand, the extract of the neem seed in the 1:12 combination with 70% ethanol was more effective on *S. frugiperda* larvae. Therefore, ethanol concentration used in this bioassay, contributes to the disposition of the biocidal properties present in the neem seeds.

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REFERENCES

Allende, R.M., P.A. Picos M., I. Márquez, Z., J.A Carrillo F., R.S. García, E. & J. León, F. (2013). Identificación morfológica y molecular de *Penicillium oxalicum* causante de pudrición de tallos y frutos de tomate. *Revista Mexicana de Fitopatología* 31: 13-19.

Amin, N. (2013). Investigation of culture filtrate of endophytic fungi *Nigrospora* sp. isolate RS 10 in different concentrations towards root-knot nematode *Meloidogyne* spp. *Indian Journal of Sciencie and Technology* 6: 5177-5181.

Ángel-Ríos, M.D., J. Pérez-Salgado & F. Morales, J. (2015). Toxicidad de extractos vegetales y hongos entomopatógenos en el gusano cogollero *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae), del maíz en el Estado de Guerrero. *Entomología Mexicana* 2: 260-265.

Barnett, H.L. & Hunter, B.B. (1998). *Illustrated Genera of Imperfect Fungi*. 4th ed. Burguess Publishing Company.

Minneapolis, MN, USA. pp. 241.

Barth, G. & C. Gallardin. (1996). *Yarrowia lipolytica*. In: Wolf, K. (ed). Non-conventional yeasts in biotechnology. A handbook. Springer-Verlag. Germany. pp. 313-388.

Berlanga-Padilla, A. M., Ayala-Zermeño, M.A., Gallou, A., Serna-Domínguez, M.G., Montesinos-Matías, R., M.A. Arredondo-Bernal, A. Gallou, M.G. Serna-Domínguez, R. Montesinos-Matías & H.C. Arredondo-Bernal. (2016). Identificación de *Lecanicillium longisporum* asociado a *Melanaphis sacchari* (Hemiptera: Aphididae), en sorgo. *Revista Mexicana de Micología* 44: 51-54.

Borges, D., A.O Díaz, A.N San Juan & E. Gómez. (2010). Metabolitos secundarios producidos por hongos entomopatógenos. *ICIDCA sobre los derivados de la caña de azúcar* 44: 49-55.

Burgos-Dueñas, O., A. Burgos-Solorio, J. Folch, M., C. Morales, H. & I. Tello, S. (2014). Identificación de hongos entomopatógenos asociados a *Pseudips mexicanus* hopkins, 1915 (Curculionidae: Scolytinae). *Entomología Mexicana* 1: 326-333.

Constanski, K.C., J. Zorzetti, P.H. Santoro, A. Thibes, H. & P.M. Oliveira, J.N. (2016). Inert powders alone or in combination with neem oil for controlling *Spodoptera eridania* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae. *Ciências Agrárias* 37: 1801-1810.

Cortez-Madrigal, H. (2006). Efecto de Coadyuvantes en *Lecanicillium lecanii* (Zimm.) Zare y Gams y su virulencia hacia *Toxoptera aurantii* Boyer. *Revista Mexicana de Fitopatologia* 24: 59-64.

Do Prado, R.L., L.E. Costa, M., C.P. D'alessandro, J. Djair, V. & I. Delalibera, J. (2014). *In vitro* compatibility of an acetogenin-based bioinsecticide with three species of entomopathogenic fun. *Florida entomologist* 97: 1395-1403.

García, G.C., M.B. González, M. & N. Bautista, M. (2011). Patogenicidad de aislamientos de hongos entomopatógenos contra *Spodoptera frugiperda* (Lepidoptera: Noctuidae) y *Epilachna varivestis* (Coleoptera: Coccinellidae). *Revista Colombiana de Entomología* 37: 217-222.

García-Gutiérrez, C., M.B González-Maldonado & E. Cortez-Mondaca. (2012). Uso de enemigos naturales y biorracionales para el control de plagas de maíz. *Ra Ximhai* 8: 57-70.

González-Maldonado, M.B., J.N. Gurrola-Reyes & I. Chaírez-Hernández. (2015). Productos biológicos para el control de *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Revista Colombiana de Entomología* 4: 200-204.

Klein, J. P. & L. Moeschberger, M. (2005). Survival analysis: techniques for censored and truncated data. Springer Science & Business Media. 536 pag. 2da edición.

Molina, N. (2001). Uso de extractos botánicos en control de plagas y enfermedades. Avances en el Fomento de Productos Fitosanitarios No-Sintéticos. *Manejo Integrado de Plagas* (*Costa Rica*) 59: 76-77.

Morales, L.A.D., R. Gálvez, J., J. Gómez, R., O. Díaz, G. & J. Marín, S. (2014). Evaluación de un formulado de aceite vegetal de *Beauveria bassiana* en condiciones de laboratorio para el control de la broca del café. *Fitosanidad* 18: 5-14.

Motta-Delgado, P.A. & B. Murcia-Ordoñez. (2011). Hongos entomopatógenos como alternativa para el control biológico de plagas. *Revista Ambiente e Agua* 6: 77-90.

Muñiz-Reyes, E., C.A. Ramos, B., C. Rodríguez-Hernández & L.D. Ortega-Arenas. (2016). Actividad biológica de nim en adultos de mosca blanca *Trialeurodes vaporariorum* (Aleyrodidae) West. *Revista Mexicana de Ciencias Agrícolas* 7: 1283-1295.

Nava-Pérez, E., C. García-Gutiérrez, J.R. Camacho-Báez & E.L. Vázquez-Montoya. (2012). Bioplaguicidas: una opción para el control biológico de plagas. *Ra Ximhai* 8: 17-29.

Nexticapan-Garcéz, A., A. Magdub-Méndez, S. Vergara-Yoisura, R. Martín-Mex & Larqué-Saavedra. (2009). Fluctuación poblacional y daños causados por gusano cogollero (*Spodoptera frugiperda* J. E. Smith) en maíz cultivado en el sistema de producción continúa afectado por el huracán Isidoro. *Universidad y Ciencia* 25: 273-277.

Lezama, R., J. Molina, M. López, A. Pescador, E. Galindo, C.A. Ángel & C.A. Michel. (2005). Efecto del hongo entomopatógeno *Metarhizium anisopliae* sobre el control del gusano cogollero del maíz en campo. *Avances en Investigación Agropecuaria* 9: 69-74.

Ramanpreet, K.S. & J.B. Michael. (2012). The insect-pathogenic fungus *Metarhizium robertsii* (Clavicipitaceae) is also an endophyte that stimulates plant root development. *American Journal of Botany* 99: 101-107.

R Core, Team. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reyes, F.D. & R. Fernández, Da S. (2013). Efecto biocida *in vitro* del extracto foliar de *Azadirachta indica* en *Staphylococcus* sp y *Pseudomonas* sp. *Salus* 17: 34-41.

Reséndiz, R.Z., J.A López, S., B. Estrada, D., E. Osorio, H., J.A Pecina, M. & M.C. Mendoza, C. (2017). Efectos genéticos de la resistencia a *Spodoptera frugiperda* en líneas de maíz derivadas de germoplasma nativo de Tamaulipas. *Revista Mexicana de Ciencias Agrícolas* 8: 1329-1341.

Rios V., C., J. C. Bustillos R., M. Ordoñez G., M. F. Ruiz C., D. I. Berlanga R., J. J. J. Ornelas P., P. B. Zamudio F., C. H. Acosta M., G. I. Olivas O., D. R. Sepúlveda A., M. A. Salas M., J. L. Jacobo C., O. J. Cambero C. & G. Gallegos M. (2017). Manual de uso y aplicación de bioinsecticidas microencapsulados para el manejo de *Spodoptera frugiperda* y *Helicoverpa zea*. SAGARPA-COFUPRO. México. pp. 56.

Rivera, P.F.A., V. González, S., J.G. González, A. & P.A. Ossa, L. (2016). Caracterización molecular, análisis morfológico y colonización micorrízica en la rizosfera del aguacate (*Persea americana* Mill) en Caldas, Colombia. *Acta Agronómica* 65: 398-405.

Silva, A.G., A. Lagunes, T., J.C. Rodríguez, M. & D. Rodríguez. (2002). Insecticidas vegetales: una vieja-nueva alternativa en el control de plagas. *Revista Manejo*

Integrado de Plagas 66: 4-12.

Sharma, L. & G. Marques. (2018). *Fusarium*, an Entomopathogen-A Myth or Reality? *Pathogens* 7: 93.

Therneau, T.M. (2018). _A package for survival analysis in S_. versión 2.42-3. https://cran.r-project.org/web/packages/survival/index.html.

Trujillo, R.P.A., L.N. Zapata, R., R.A. Hoyos, S., F.C. Yepes, R., J. Capataz, T. & F. Orozco, S. (2008). Determinación de la DL50 y TL50 de extractos etanólicos de suspensiones celulares de *Azadirachta indica* sobre *Spodoptera frugiperda. Revista Facultad Nacional de Agronomía* 61: 4564-4575.

Trujillo, G.Z., R.P. Pérez, D. Borroto & E. Concepción. (2003). Efectividad de hongos entomopatógenos y *Bacillus thuringiensis* sobre *Thrips palmi* Karny en el cultivo del pepino. *Fitosanidad* 7: 13-18.