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SCIENTIFIC ARTICLE
Plant Health

Entomopathogenic nematodes for the control of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) pupae

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ABSTRACT
Helicoverpa armigera (Hübner) is a polyphagous insect of difficult control and maize is an important host crop of this insect. Entomopathogenic nematodes (EPNs) are control agents of soil pests. This study aimed to verify the action of EPNs for the control of *H. armigera* pupae. Laboratory and greenhouse bioassays were conducted to select the concentration of nematode application and subsequently field test were conducted. It was obtained that *Heterorhabditis amazonensis* MC01 at the concentration of 400 infective juveniles (IJs) ·pupa^{-1} caused the highest mortality in a lower concentration, whereas for *H. amazonensis* JPM4, concentrations of both 200 and 400 IJs ·pupa^{-1} were similar causing pupae mortality. In the greenhouse, *H. amazonensis* MC01 caused mortality reached values of 80% after 10 days, at concentrations of 600 and 800 IJs ·pupa^{-1}. The highest mortality caused by *Steinernema carpocapsae* was observed at eight days after the juvenile application, at a concentration of 600 IJs ·pupa^{-1}, also reaching 80% mortality. In the field test, both forms of application were considered appropriate for *H. amazonensis* MC01, causing mortality rates of up to 80%.

Keywords: biological control; Heterorhabditidae; Steinernematidae; *Zea mays*.

INTRODUCTION

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is a cosmopolitan pest that feeds on at least 170 species of plants, including important crops, since cotton, soybean and beans (JHA et al., 2012). Maize (*Zea mays* L.) is one of the main hosts of this insect. The caterpillars prefer to feed on the shoots and reproductive organs of plants, resulting in high annual costs spent on control, besides production losses that reach US$ 5 billion (LAMMERS; MACLEOD, 2007; CZEPAK et al., 2013). Other characteristics of this species are its high mobility, fecundity and ability to survive. These characteristics favored the establishment of this insect in Brazil, where it has easily reached the status of pest and has become a major phytosanitary problem in several maize-producing regions of the country (ÁVILA et al., 2013).

According to the Brazilian Ministry of Agriculture, Livestock and Supply, there are 44 chemical and biological insecticides registered for the control of *H. armigera* in soybean, including emamectin benzoate, that was not registered

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in the country until 2017 (MAPA, 2020). However, in different parts of the world, cases were found of *H. armigera* resistance to the main insecticides used for its control. Thus, it is fundamental to associate different control methods to reduce selection pressure on the pest (KAMARAJ et al., 2008).

About 30% of the insecticides used worldwide are directed to this species, mainly due to the high levels of resistance (UDAMALE et al., 2013). *Helicoverpa armigera* has a great ability to develop resistance to insecticides, and also an ability to develop resistance to Cry proteins introduced in genetically modified plants, which are toxic to larvae (AKHURST et al., 2003). In this way, different strategies should be considerate to control *H. armigera*.

Biological control using entomopathogenic nematodes (EPNs) can be effective to assist in the control of *H. armigera*. Different studies report the use of EPNs for the control of prepupae and pupae of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in maize, reaching high mortality rates (FEASTER; STEINKRAUS, 1996; ANDALÓ et al., 2010). As these insects present a habit of life similar to *H. armigera*, it is important to study EPN as a biological control agent of this pest.

Nematode species, such as *Heterorhabditis amazonensis* Andaló, Nguyen and Moino Jr. (ANDALÓ et al., 2006) and *Steinernema brazilense* Nguyen, Ginarte, Leite, Santos and Harakava, have been described in Brazil (NGUYEN et al., 2010). The use of native species is closely related to the success of control programs due to the adaptation of the nematode to the environment and the specificity to the target insect. Entomopathogenic nematode can be also effective to target insects living in the soil or in cryptic environments (ARTHURS et al., 2004), which is the same condition where *H. armigera* pupae remain.

Considering the potential of EPNs in the control of *H. armigera* pupae and the possibility of using native populations adapted to local conditions, this study aimed to verify the action of EPNs for the control of *H. armigera* pupae in laboratory, greenhouse and field conditions.

MATERIAL AND METHODS

The establishment of *H. armigera* in laboratory was performed according to the methodology described by VILELA et al. (2014).

To obtain recently emerged infective juveniles (IJs), the populations stored in aqueous suspension in a climate chamber at 16 ± 2 °C were multiplied in larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) raised according to the POTRICH et al. (2007) methodology. The larvae that died with symptoms of infection were washed with water and placed in a dry chamber (Petri dish with filter paper) for 5 days. After that, the larvae were removed and placed in white traps to collect the IJs. The infective larvae were kept in a climate chamber at 26 ± 2 °C. Infective juveniles collected for up to 3 days after emergence were used in experiments.

Entomopathogenic nematodes concentration: laboratory bioassay

Helicoverpa armigera pupae that were up to 5 days old were used in the experiment. Concentrations of 100, 200, 400 and 600 IJs per pupa were tested. As control, only water was applied. The nematodes used were *H. amazonensis* MC01 (population isolated in Monte Carmelo, MG, Brazil) and *H. amazonensis* JPM4 (population isolated in Lavras, MG, Brazil).

The IJs suspensions were added to Petri dishes (9 cm in diameter) containing Plantmax substrate (12.3 g) with five *H. armigera* pupae displaced partially buried. The applied suspension volume was the same for all treatments, 6 ml, in order to keep the substrate moist. Five replicates were performed per treatment in a completely randomized design. Evaluations of pupa mortality were performed after 5 days of IJs application. The dead pupae were kept in a dry chamber for 4 days for subsequent dissection and confirmation of mortality caused by the nematode. The experiment was conducted in a climate chamber at 26 ± 2 °C and 12 h photophase. The data were submitted to analysis of variance and the means adjusted by the regression analysis ($p < 0.05$), after the assumptions were met.

Greenhouse bioassay

The experiment in greenhouse tested the species selected from the concentration test and included the nematode *Steinernema carpocapsae* All (Weiser) (from Florida, USA) as a comparison, based on the nematode behavior, since

S. carpocapsae presents a habit known as ambusher, while *H. amazonensis* is considered a cruiser (WILSON et al., 2012; ANDALÓ et al., 2017), and these characteristics can affect the success of pest control.

Five pupae of *H. armigera* aged up to two days were placed in a plastic pot (1.4 L 10 cm in diameter) containing sterilized soil classified as dystrophic red latosol. The pupae were buried approximately 3 cm deep in the soil, and a suspension of 40 mL of IJs of *H. amazonensis* and *S. carpocapsae* at the concentrations of 200, 400, 600 and 800 IJs ·pupa^{-1} was applied. To the control was applied only water. Five replicates were performed per treatment, and the evaluations were performed 2, 4, 6, 8 and 10 days after the establishment of the experiment. To confirm mortality, the pupae were dissected and observed under a stereomicroscope to confirm the presence of nematodes. The data were submitted to analysis of variance and the means adjusted by the regression analysis ($p < 0.05$), after the assumptions were met.

Field bioassay

The experiment was conducted in an experimental area in the domain of Plateau and Tablelands of the Paraná Sedimentary Basin (18°43'31"S, 47°31'32"W, 890 m of altitude) in the southwest portion of the Brazilian Cerrado biome. The climate of the region is of Cwa type, according to Köppen classification. The soil of the area is classified as dystrophic red latosol, a moderate, medium texture, tropical Cerrado subdeciduous and mild wavy relief type.

Hybrid maize 'SHS 7920 Santa Helena' seeds were sown, spacing 50 cm between rows, plant density of 62,000 plants ha^{-1}. The herbicides glyphosate, atrazine and 2,4 D, and chlorpyrifos insecticide for control of *S. frugiperda* were used at 40 days after plant emergence. The fertilization was carried out using 120 kg ·ha^{-1} of N (30 kg ·ha^{-1} at planting and 90 kg ·ha^{-1} coverage at V4 development stage), and at planting 80 kg ·ha^{-1} P_2O_5 and 60 kg ·ha^{-1} K_2O were also used.

The experimental area was approximately 300 m². The experiment was installed when the maize was at the R4 stage, about 30 days after pollination. The nematode tested was *H. amazonensis* MC01. Two treatments were used: the application of the aqueous suspension of nematodes and the release of nematodes via *T. molitor* cadavers. For the control, the nematode was not applied.

Tenebrio molitor cadavers containing the IJs were released every m^2. For treatment with suspension, 80,000 IJs in 1 L of suspension was applied per m^2 with pressurized sprayer with CO_2. Evaluations were done daily, for 5 days, starting 4 days after nematode release in the field.

The experimental design was a completely randomized block in subdivided plots; the first factor was the treatments and the second factor the evaluated days. Eight blocks were used, totaling 24 plots of 12 m^2. Each plot received 10 pupae of *H. armigera* of up to 2 days-old that were buried at 3 cm depth and placed along the lines in the plot. Each place where the pupae were inoculated was marked for the subsequent evaluations.

The evaluated data were based only in *H. armigera* pupae mortality. None data of production were obtained, since it was not the objective of this study. Mortality data were submitted to analysis of variance, and the means were compared by the Tukey's test ($p < 0.05$) and regression analysis, after the assumptions of the model were met.

RESULTS AND DISCUSSION

Entomopathogenic nematodes concentration: laboratory bioassay

Regarding concentration of IJs suspension to control *H. armigera* pupae, it was verified that mortality showed sigmoidal behavior, with a positive correlation between concentration mortality for *H. amazonensis* MC01. The dosage of 100 IJs ·pupa^{-1} was not efficient and did not differ from the control. Both concentrations of 400 and 600 IJs ·pupa^{-1} caused 45% mortality (Fig. 1). The lower concentration for spraying, 400 IJs ·pupa^{-1}, was selected since it can offer a better ratio between economy and efficacy.

Considering *H. amazonensis* JPM4, the mortality followed exponential growth, and the concentration of 200 IJs ·pupa^{-1} was enough to cause 40% mortality in the insects. There was a reduction in the percentage of pupal mortality with increasing concentrations from 400 to 600 IJs ·pupa^{-1}. This contrasted with what occurred with *H. amazonensis* MC01, which presented an increase in pupal mortality rates associated with an increase in the concentration, and an establishment at the higher concentrations (Fig. 1).

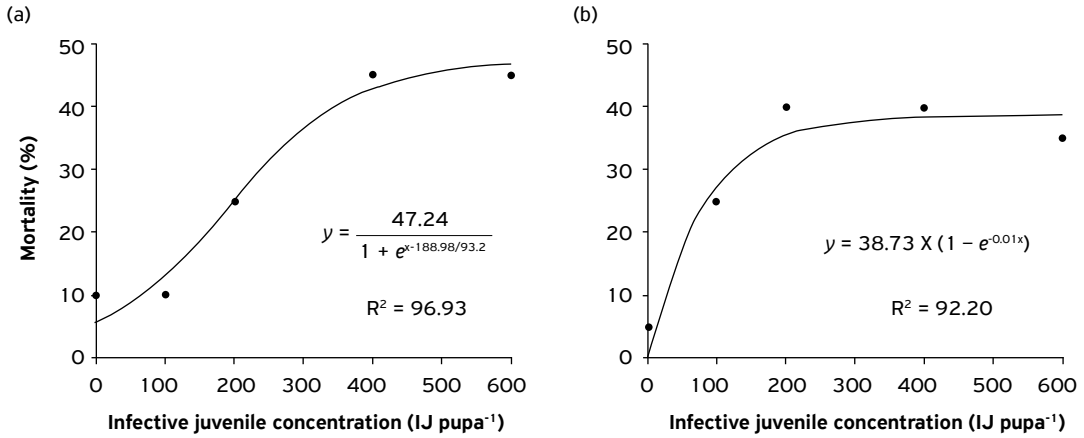

Figure 1. Mortality of *H. armigera* pupae caused by different concentrations of *H. amazonensis* MCO1 (a) and *H.amazonensis* JPM4 (b) under laboratory conditions (26 ± 2°C and 12 h photophase).

Greenhouse bioassay

According to the mortality rates of *H. armigera* pupae in a greenhouse using different concentrations of *H. amazonensis* MC01, there was a higher mortality of pupae as the concentration of IJs increased. Mortality reached values of 80% after 10 days, at concentrations of 600 and 800 IJs. Thus, the lowest number of nematodes that caused a high mortality was considered the concentration of 600 IJs. It was also observed that from the sixth to the eighth day there was an increase in mortality, and on the tenth day there was low incidence of dead pupae. The action of the nematode on the pupae occurred mainly between 6 and 8 days after the inoculation of the infective juveniles (Fig. 2).

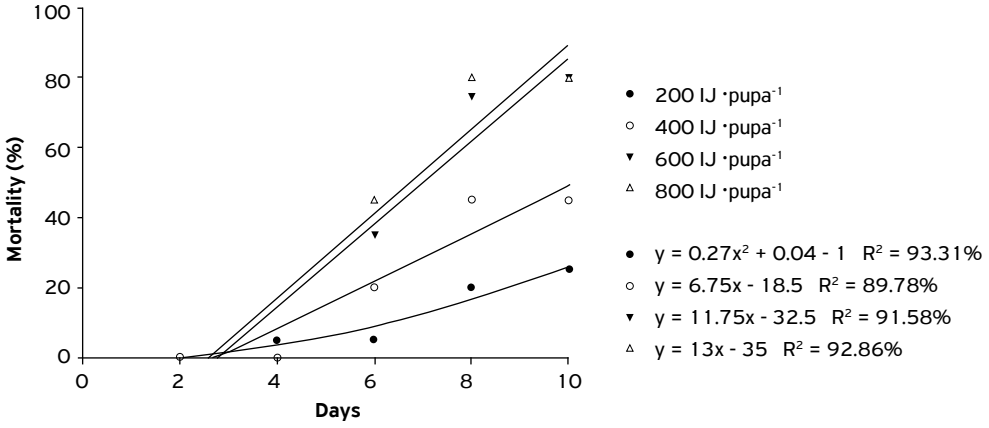

Figure 2. Mortality of *H. armigera* pupae using different concentrations of *H. amazonensis* MCO1 under greenhouse conditions.

Considering *S. carpocapsae* All, there was an increment in mortality as the concentration of IJs increased. There was also an increase in pupal mortality from the sixth day of evaluation. Thus, at the highest concentrations tested, 600 and 800 IJs, there was the highest pupal mortality rate. The highest mortality was observed at 8 days after the juvenile application, at a concentration of 600 IJs (reaching 80% mortality). This percentage of mortality was also obtained at the concentration of 800 IJs, but only 10 days after the IJs inoculation (Fig. 3).

Field bioassay

The interaction between the treatments and days was significant ($p < 0.01$). A gradual increase occurred in the mortality of *H. armigera* pupae caused by *H. amazonensis* MC01 over time. From the second day of evaluation, there was an increase in pupal mortality, which stabilized on the fifth day of evaluation. In the two initial evaluations, treatment with IJs suspension caused higher mortality than treatment with *T. molitor* cadaver. However, in the subsequent evaluations, these treatments were similar, with 77 and 75% of pupal mortality, respectively, in the last evaluation (Fig. 4). In the control, pupal mortality reached a maximum value of 15%, related to environmental factors and adults that had emerged.

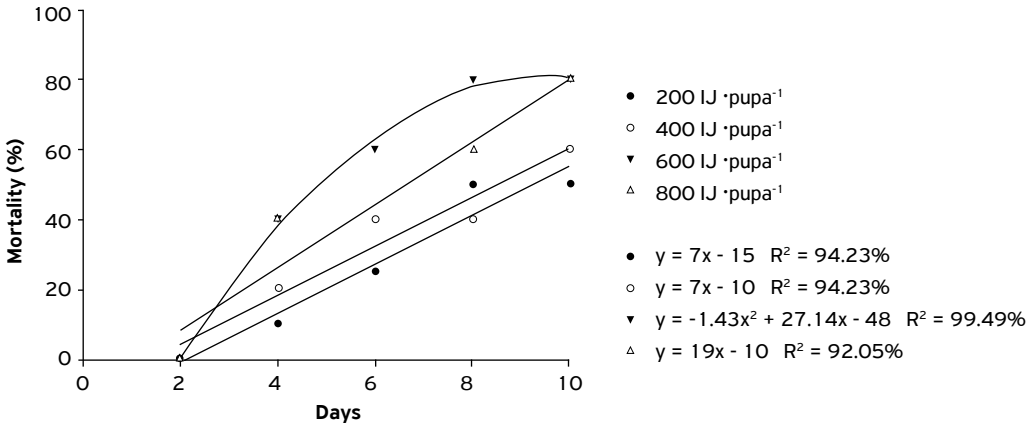

Figure 3. Mortality of *H. armigera* pupae using different concentrations of *S. carpocapsae.* All under greenhouse conditions.

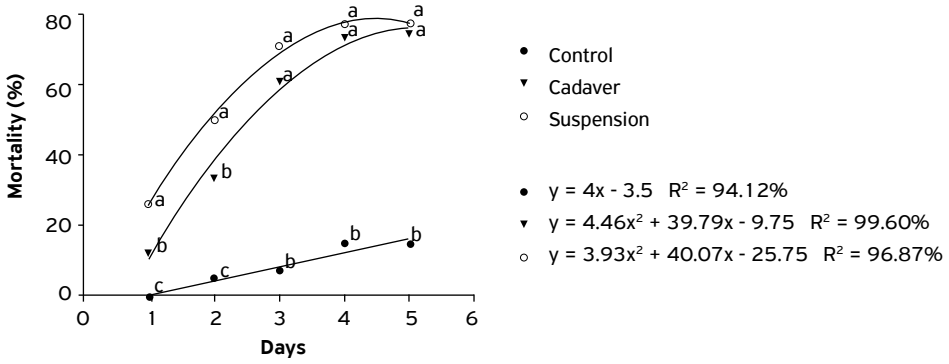

Figure 4. Mortality of *H. armigera* pupae caused by *H. amazonensis* MCO1 under field conditions. Same letters associated to the observed means for each day indicate that the treatments do not differ by Tukey test at 5% probability.

DISCUSSION

Considering studies with species of similar behavioral habits in the pupal phase and close taxonomic category, it is possible to make inferences with studies developed with other Noctuidae, such as *S. frugiperda*. The virulence of *S. frugiperda* larvae was studied by FUXA et al. (1988), and it was observed that there were variations in mortality caused by different populations of the same nematode species, *Steinernema feltiae* (Filipjev).

MOLINA-OCHOA et al. (1996) affirmed that *S. carpocapsae*, *Steinernema riobravis* Cabanillas, Poinar and Raulston, and *Heterorhabditis megidis* Poinar, Jackson and Klein were nematodes considered potential in the control of *S. frugiperda*, while ANDALÓ et al. (2010) obtained higher mortality rates using the nematodes *S. arenarium* A11 (Artyukhovsky) and *H. amazonensis* RSC02. CACCIA et al. (2014) verified under laboratory conditions that *Steinernema diaprepesi* Nguyen and Duncan caused mortality rates to larvae of *S. frugiperda* from 93 to 100% and 87 to 93% for *Helicoverpa gelotopoeon* (Dyar).

In addition, mortality rates could also vary depending on the nematode populations tested and the susceptibility of the insect population. According to RAULSTON et al. (1992), prepupae and pupae of *S. frugiperda* suffered 46% mortality when using *Steinernema* sp.; whereas MOLINA-OCHOA et al. (1996) obtained pupae mortality rates of at most 43%. These indices are lower than those found for larvae, which can be attributed to the difficulty experienced by nematodes when trying to penetrate the pupae. However, few studies were conducted using entomopathogenic nematodes on pupae of *H. armigera*.

MOLINA-OCHOA et al. (1996), studying the efficiency of different nematode species to control *S. frugiperda*, obtained data showing that *H. bacteriophora* caused mortality of 64.7% in caterpillars of this species at a concentration of 100 IJs $\cdot mL^{-1}$, but with low mortality in pupae (5.7%). Thus, it is important to select the correct concentration of isolates of these entomopathogenic organisms in order to avoid possible failures in treatments or unnecessary expenses.

According to ALVES et al. (2009), it is very important to conduct studies with nematode concentrations in the laboratory, due to the fact that the use of EPNs only tends to be economically viable when smaller concentrations are required, generating a lower final cost for nematode production.

Therefore, considering that both nematodes *H. amazonensis* JPM4 and *H. amazonensis* MC01 were virulent to *H. armigera* pupae, *H. amazonensis* MC01 was selected for further tests, since it is a native population of the study region, isolated in the municipality of Monte Carmelo, MG, Brazil, already adapted to the local environmental conditions.

SOUZA et al. (2012) developed experiments in greenhouse conditions and obtained that at concentrations of 150, 300 and 600 IJs ·insect^{-1} *H. amazonensis* RSC05 caused mortality in *S. frugiperda* similar to that found in the tests with *H. armigera*, reaching values close to 80%, corroborating the data obtained in the present study.

According to ALI et al. (2007, 2008) *Steinernema seemae* Ali, Shaheen, Pervez and Hussain applied for control of *H. armigera* larvae and prepupae was promising as a pest control agent, due to the high mortality rates observed. NAVON et al. (2002) have demonstrated the potential of nematodes application in the field, since they can remain in the soil for a long period of time, which could continue causing mortality of the pest for several years of cultivation.

CONCLUSION

Heterorhabditis amazonensis MC01 at the concentration of 400 IJs ·pupa^{-1} promoted higher mortality in *H. armigera* pupae. *Heterorhabditis amazonensis* MC01 and *S. carpocapsae* All were effective in controlling *H. armigera* pupae at concentrations of 600 IJs ·pupa^{-1} under greenhouse conditions. *Heterorhabditis amazonensis* MC01 was effective causing mortality to *H. armigera* pupae in the field when applied as an aqueous suspension and using the *T. molitor* cadaver under the tested conditions.

AUTHORS' CONTRIBUTIONS

Conceptualization: Andaló, V.; Assis, G.A.; Faria, L.S. **Data curation:** Andaló, V.; Assis, G.A. **Formal analysis:** Carvalho, F.J.; **Funding acquisition:** Andaló, V.; Assis, G.A.; Santos, V.; Mendes, S.M.; Gonring, A.H.R. **Methodology:** Andaló, V.; Assis, G.A.; Faria, L.S. **Project administration:** Andaló, V.; **Writing – review & editing:** Andaló, V.; Assis, G.A.; Faria, L.S.; Carvalho, F.J.; Santos, V.; Mendes, S.M.; Gonring, A.H.R.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this published article.

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CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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