IJAMBR 4 (2016) 60-65 ISSN 2053-1818

International Journal of Applied Microbiology and Biotechnology Research
www.bluepenjournals.org/ijambr

Loss caused by fruit worm and its treatment with entomopathogenic nematodes in fruits of *Physalis ixocarpa* Guenee

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Article History
Received 30 June, 2016
Received in revised form 31 July, 2016
Accepted 08 August, 2016

Keywords:
Entomopathogenic nematodes,
***Heliothis subflexa*,**
***Physalis ixocarpa*.**

Article Type:
Full Length Research Article

ABSTRACT

Under laboratory and field conditions, the loss in fruits of tomatillo (*Physalis ixocarpa*) caused by the larvae of *Heliothis subflexa* Brot in different instar/week was determined. As a control method in the laboratory, the effects of entomopathogenic nematodes (*Heterorhabditis bacteriophora, Steinernema carpocapsae, Steinernema feltiae* and *Steinernema westeri*) in three concentrations (200, 400 and 600 nematodes/larvae, respectively) in the fruit worm's larvae (*Heliothis subflexa*) was evaluated. In the field, four treatments (*H. bacteriophora, S. feltiae*, Thimet® and Witness) were also evaluated. The strains of entomopathogenic nematodes were applied in a concentration of 500 nematodes/plant. The average loss found in fruits per day in the larvae from the 1st instar was 0.128 g; from the 2nd instar, it was 0.217 g; and from the 3rd instar, it was 0.29 g. Out of 799 fruits studied, 65.5% was found to have larvae from different instar. In the laboratory, the mortality rate of *H. subflexa* larvae in the 3rd instant after 24 h of infestation by the nematode *S. carpocapsae* with densities of 200 and 600 nematodes/larvae respectively, were 80 and 100%, respectively. At the same duration and densities, using *S. westeri* as the nematode of infestation, the mortality rate were 66.6 and 100%, respectively. In the field, it was found that the best treatment was with Thimet, with a performance of 386.56 kg; followed by the treatment with *H. bacteriophora* nematode (318.88 kg). In the Witness, a performance of 209.28 kg was obtained in 80 plants/treatment.

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INTRODUCTION

The growing of tomatillo, also called “green tomate” or “tomateoffresadilla” (*Physalis ixocarpa* Brot.), is important in Mexico. This species is distributed all along the Mexican Republic in a great diversity of environments. That is why it is 3500 m above sea level until above sea level (Santiaguillo et al., 2012). *Heliothis subflexa* (fruit worm) is a monophagous insect that attacks species from the genus *Physalis*. In early stages of the development of the fruit, the insect feeds itself out of these species and moves along looking for more fruits to complete its development, the attacked fruits generally fall down. The adults are nocturnal moths which lay their cocoons in the young leaves and when the larvae comes out, it begins to eat the leaves to penetrate the fruits afterwards, turning them rotten (Morales et al., 2007). The importance of growing “tomatillo” in Oaxaca is really high because of the loss in crops and the negative economic impact on the incomes of the regional producers; that is why it is considered as a serious study to find out the reason for loss

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and damage caused by the main pests and diseases. Also, to find out the most effective control methods, such as the case of *H. subflexa*. Albert (1998), stated that the greatest effort to kill off the pests and diseases is more focused on the systemic chemical control, which leaves toxic residues in the agricultural products, affecting the feeding security of the population, and at the same time it causes pollution, and the toxicity to the producer, and the pests' resistance to agricultural pesticides. Aquino (2003) suggested that a biological control is a potential alternative for the economically important pests; especially microbial control, due to their development habits. This is because, either as larvae or adults, they are susceptible to infections caused by microorganisms such as fungi and entomopathogenic nematodes that can be used in this kind of control. One of the alternatives is biological control. This is a pest control strategy that uses parasites, predators and pathogens to control pest infestation.

The parasitic nematodes are used as potential agents for insect pests plague control. Biological control agents could offer friendlier environmental alternatives than pesticides do. They may also be used when the pest has developed resistance to conventional pesticides. That is, biological pesticides are the most selective control agents according to Girón-Pablo et al. (2015) and Alatorre (2008).

The objective of this research was to determine the real loss caused by larvae from different instar of *H. subflexa,* and the treatment with *S. feltiae*, *S. carpocapsae*, *S. westeri* and *H. bacteriophora* nematodes under laboratory and field conditions.

MATERIALS AND METHODS

This study was evaluated under laboratory and field conditions, both experiments were done at the Interdisciplinary Center of Investigation for the Integral Regional Development Unit, Oaxaca, National Polytechnic Institute (CIIDIR-Oaxaca IPN), México. The kind of "tomatillo" evaluated was the so called Diamante.

Loss in fruits of *P. ixocarpa* done by larvae of *H. subflexa* in 1st, 2nd and 3rd instars, under laboratory conditions

To determine the loss in fruits of *P. ixocarpa*, a fruit and a larva of 1st instar was set in 100 mL container. The fruit was weighted every day, and these fruits were changed once a week. The weight control was done for eight weeks. The losses caused by 2nd and 3rd instar larvae were observed under the same procedure.

For this assessment, 100 larvae per instar (1st, 2nd and 3rd) were used; totaling 300 larvae.

Loss in fruits of *P. ixocarpa* caused by *H. subflexa* larvae in the field

To determine the real reason why the fruits were falling down, that is, whether the loss and damage in fruits were caused by *H. subflexa* or by wind factor, the fruits of *P. ixocarpa* plant on the floor were collected per evaluated treatment [Witness, Thimet®15G. Forato: 0,0-dietil-S (etiltio)-metilfosforoditioato, *S. feltiae.* and *H. bacteriophora*). The gathering of fruits was done in a periods of 8 days from the presence of fruits on the floor, which were taken to the entomology laboratory and were set in transparent containers of 100 mL and observed for a period of 30 days.

Controlling *H. subflexa* larvae using entomo pathogenic nematodes, performed under laboratory conditions

Four strains of entomopathogenic nematodes in three different concentrations; (200, 400 and 600 nematodes/ larvae) were evaluated. The entomopathogenic nematodes evaluated were *H. bacteriophora*, *S. carpocapsae*, *S. feltiae* and *S. westeri*. In plastic containers of 100 mL, a larva from 3rd instar was set, so that they feed themselves from the mature fruit of *P. ixocarpa*. The application of the nematodes was done directly in the fruits where the *H. subflexa* larvae feed themselves. The observations were performed every 8 h. The experiment was ended when 100% of the larvae were dead. Seven replicates were performed per treatment (Figure 1), and the DL_{50} which was used as a control method in the field were determined.

Controlling *H. subflexa* larvae using entomo pathogenic nematodes, performed under field conditions

In the field, four treatments were performed; two of them been entomopathogenic nematodes (*H. bacteriophora, S. feltiae*, Thimet®15G and Witness), the entomopathogenic nematodes' strains were applied to a concentration of 500 nematodes/plant and Thimet® to a concentration of 1 L/1000 of water. The treatments were applied three times in the crop in intervals of two weeks. The first application was done when the plant was 40-50 cm high (the blooming time). For the study, completely random design was the experimental design; with four treatments and four replications per treatment in padded plastic.

Statistical analyses

Two-way ANOVA was performed for analyzing the results

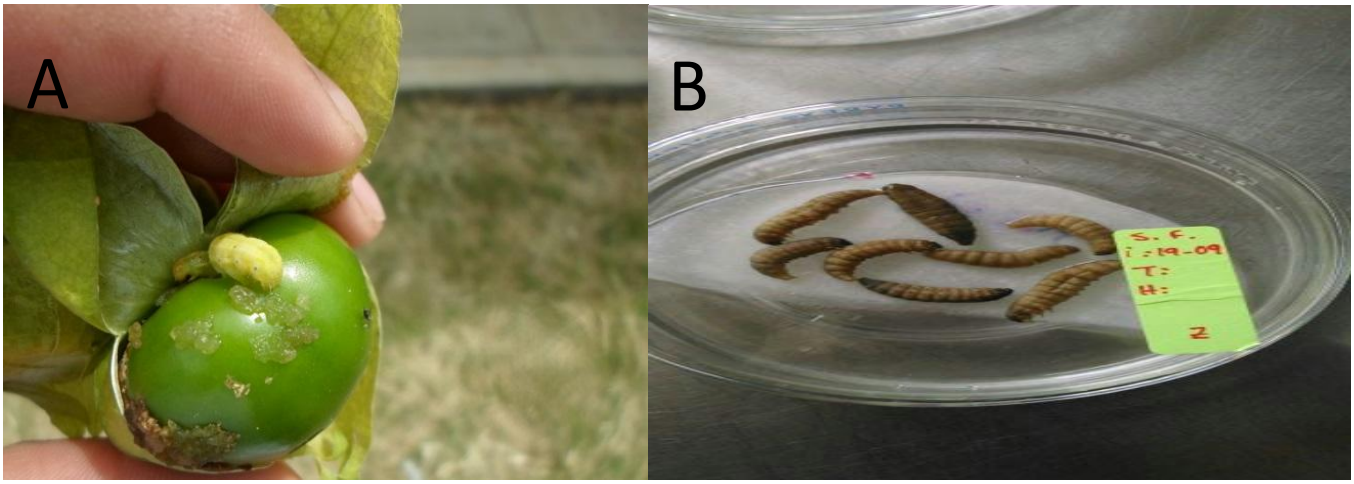

Figure 1. A) *H. subflexa* larvae from 3rd instar extracted from fruits of *P. ixocarpa*; **B)** dead larvae in humid beds by the effect of entomopathogenic nematodes.

Table 1. Loss in fruits of *P. ixocarpa* caused by *H. subflexa* larvae from 1st, 2nd and 3rd instars; and the average weight per week (g).

Larvae	Weeks								Total loss
Stages	1. [a]	2. [a]	3. [a]	4. [a]	5. [a]	6. [a]	7. [a]	8. [a]	
1st-2nd	1.11 a	0.7 a	0.69 a	0.76 a	1.03 a	0.81 a	1.0 a	0.90 a	7.0 a
2nd-3rd	1.23 a	1.82 a	1.86 a	1.40 a	1.45 a	1.13 a	1.78 a	1.52 a	12.19 b
3rd-coccon	2.75 ab	2.35 ab	2.40 ab	2.33 ab	2.15 ab	2.28 ab	2.37 ab	2.03 ab	18.14 c

Means with the same letter are not statistically significant (P<0.05).

obtained. Also, Tukey's multiple ranks test at 95% of probability was employed. The statistical software used was Analysis System (SAS, 2002), which determined the performance among the different treatments assessed.

RESULTS AND DISCUSSION

Observations on the loss in fruits of *P. ixocarpa* caused by *H. subflexa* larvae from 1st, 2nd and 3rd instars done under laboratory conditions

The loss of fruits per day was 0.128 g in the 1st instar larvae stage. From the 2nd instar larvae stage, it was 0.217 g while from the third instar larvae stage, it was 0.29 g. There was no significant difference (P>0.05) per week in the loss caused by *H. subflexa* larvae from the 1st, 2nd and 3rd instars (Table 1). In a period of 8 weeks, an average loss of 7 g was recorded in 1st instar larvae, an average loss of 12.19 g was recorded in 2nd instar larvae, and an average loss of 18.14 g was recorded in 3rd instar larvae. There was a significant difference (P<0.05) in the final average loss. It can be concluded that whenever there are larvae from 2nd and 3rd instars, a control method should be applied, otherwise the damage could be considerably worse as can be observed in Table 1.

Observations on the loss in fruits of *P. ixocarpa* caused by *H. subflexa* larvae done in field

A total of 799 fruits were collected from the floor and 65.6% of them were with larvae from different instar stages. However, 34.5% of them were asymptomatic of damage due to the presence of *H. subflexa.* This means that there were some physical factors (such as wind), or physiological response (due to deficiency of nutrients), that caused the falling of some fruits.

From the 524 (65.6%) fruits with the presence of treatment larvae, it was found that Witness infested the highest number of collected fruits (20.83%). The nematode *H. bacteriophora* infested 15.72% of collected fruits. While the nematode *S. feltiae* infested 16.98% of collected fruits. Lastly, Thimet® infested only 12% of collected fruits. There was a statistical difference

Table 2. Average loss due to the presence of *H. subflexa* larvae in the treatments established as control methods.

Treatment	Loss in fruits caused by *H. subflexa* (%)
Witness	20.83 a
Thimet	12 b
H. bacteriophora	15.72 a b
S. feltiae	16.98 a b

Means with the same letter are not statistically significant (P<0.05).

Table 3. Mortality of *H. subflexa* larvae with entomopathogenic nematodes done under laboratory conditions.

Treatment	24 h			48 h			72 h		
	200	400	600	200	400	600	200	400	600
H. bacteriophora	50 b	16.6 b	33.3 b	100 a	66.6 b	100 a	100 a	100 a	100 a
S. feltiae	50 b	33.3 b	16.6 b	83.3 a	83.3 a	100 a	100 a	100 a	100 a
S. carpocapsae	83.3 a	50 a	100 a	100 a	83.3 a	100 a	100 a	100 a	100 a
S. westeri	66.6 a	66.6 a	100 a	100 a	66.6 b	100 a	100 a	100 a	100 a

Means with the same letter are not substantively different (Tukey 0.05).

(P<0.05) only between the Witness and the treatment with Thimet® (Table 2). Bado et al. (2005) reported loss in *P. ixocarpa* plants caused by *H. subflexa* from 23 to 50%, even though there was no control method. The researchers set some alternative hosts such as *Physalis peruviana* as a control of *H. subflexa.*

Bravo-Mosqueda (1998) determined some loss in Central Valleys of Oaxaca, from which 50% were caused by *H. subflexa* larvae in *P. ixocarpa.* They then proposed the release of Trichogramma. Carreón (1975) reported some damage caused by *H. subflexa* of 70% from the production in Morelos. They also mentioned that the insect appeared at the beginning of the blooming; when the larvae feed themselves initially from the leaves and then from the fruit. Jiménez Gómez et al. (1992) studied *H. subflexa* for a period of 67 days, and found that this insect appeared as an adult in the flowers and as larvae in fruits of different sizes.

Observations of controlling *H. subflexa* larvae using entomopathogenic nematodes done under laboratory conditions

Under a maximum temperature of 23±2.16°C, a minimum temperature of 21±2°C; a maximum relative humidity of 56±8.36% and a minimum of 45±8.68%; the mortality of *H. subflexa* found in the larvae from the 3rd instar after 24 h treatment with 200 and 600 nematodes/larvae of *S. carpocapsae* were 83.3 and a 100%, respectively. Under the same conditions and nematode densities per fruit but with *S. westeri* as the nematode treatment, 66.6 and 100% motality were obtained, respectively. Statistically, the two treatments were significantly different (P<0.05) from the treatments with *H. bacteriophora* and *S. feltiae* nematodes. All the evaluated treatments had percentages of control higher than 83.3% and with no statistical difference in the assessed treatments. At this same time, with 400 nematodes/larvae there were 66.6 and 83.3% effectiveness and with 600 nematodes/larvae there was 100% effectiveness in all the treatments, and after 72 h, 100% effectiveness was found in all the treatments (Table 3).

Observations of controlling *H. subflexa* larvae using entomopathogenic nematodes under field conditions

The treatment, which showed the highest performance was the Thimet® with 96.72 kg; followed by the treatment with *H. bacteriophora* nematodes (79.18 kg), *S. feltiae* (60.16 kg) and lastly, the Witness (52.32 kg).

Based on the results obtained, *H. bacteriophora* nematode can be considered as a good alternative at 500 nematodes/plant in the production of *P. ixocarpa* because its performance is very close to the ones obtained with Thimet® and are very superior to the ones obtained with Witness with 31 kg more.

Statistically, only treatments with the chemicals are different from the Witness. Based on the number of fruits, treatments with the chemical and the biological treatment are similar but are different from the Witness (Table 4).

Table 4. Performance of *P. ixocarpa* (Diamante) cultivated with plastic padded.

Treatment	performance in 1/plant (kg)	Performance in 80 /plants (kg)	Number of fruits
Thimet	1. 280 a	96.72 a	140 a
H. bacteriophora (500 nematodes/plant)	0.996 ab	79.18 ab	132 b
S. feltiae (500 nematodes/plant)	0.752 ab	60.16 ab	120 b
Witness	0.654 b	52.32 b	101 c

Values with different letters in the columns indicate a significant difference for Tukey test.

Pozo (2007) reported that *Heterorhabditis* spp., under laboratory conditions strains P2M and CUH1 to 125, 250, 500 and 1400 nematodes/mL in *Heliothis virescens,* showed susceptibility in the 1st and 2nd instar larval of this insect which were the most susceptible with a 100% mortality after 24 h. Mayra et al. (2012) with *Heterorhabditis* sp. in larvae from 3rd instar of *H. virescens* in a concentration of 20 nematodes/larvae after 72 h reported that the mortality was 100% effective.

With a padded system and a dropping watering, the performance can rise from 60 to 80 t ha, much lower than the one obtained in this research, because with the *H. bacteriophora* nematode to 500 nematodes/plant, it can reach a performance of 34.5 t ha (López et al., 2009).

Liliana-Melo et al. (2010) Indicated that Scarab larvae (Coleoptera: Melolonthidae) are important pests in a diversity of crops and climates owing to their damage to the quality and yield of agricultural products. The infective ability and mortality of isolates of *Steinernema* (*S. riobravis, S. carpocapsae, Steinernema* sp., *S. arenarium*, and *S. feltiae* and *H. bacteriophora*) were determined at a concentration of 10,000 IJs/mL. The evaluation was done on larvae that were one, two and three months old. The most ineffective isolates were those of *Heterorhabditis* (93.75%).

Currently, there are very few references for control comparison in *H. subflexa* larvae with the gender of *S. carpocapsae*, *S. feltiae* and *S. westeri* nematodes, the results obtained in this study are similar to the percentage of effectiveness showed by other researchers with the gender of *Heterorhabditis* nematode.

Conclusion

P. ixocarpa fruits can tolerate larvae from 1st and 2nd instar during one week, which is a good time to apply a control with entomopathogenic nematodes (*S. feltiae* and *H. bacteriophora*) or with the Thimet pesticide.

From the total fruits that dropped, 65.5% was found to possess *H. subflexa* larvae while 34.4% is attributed to other causes. In fruits of *P. ixocarpa* the treatments with Thimet are the ones with the best results obtained, a great alternative is the use of entomopathogenic nematodes (*S. feltiae* and *H. bacteriophora*), which in those treatments have 23 and 24% of dropped fruits, respectively.

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