Bulletin of Insectology ***71*** *(2): 201-209, 2018*
ISSN 1721-8861

Interaction of *Beauveria bassiana* strain HPI-019/14 and *Bacillus thuringiensis* strain GP139 for the biological control of *Bemisia tabaci* in strawberry

Carlos Eduardo SOMOZA-VARGAS[1], Víctor Manuel HERNÁNDEZ-VELÁZQUEZ[2], Guadalupe PEÑA-CHORA[3], Gerardo TORRES-GARCÍA[4], Arturo HUERTA-DE LA PEÑA[5], Luis Daniel ORTEGA-MARTÍNEZ[1], Jesús Antonio SALAZAR-MAGALLÓN[6]

[1]*Facultad de Biotecnología-Universidad Popular Autónoma del Estado de Puebla, Mexico*
[2]*Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico*
[3]*Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico*
[4]*Centro de Investigación en Alimentos y Desarrollo, Nayarit, Mexico*
[5]*Laboratorio de Control Biológico-Colegio de Posgradudos, Puebla, Mexico*
[6]*División de Procesos Naturales, Licenciatura en Desarrollo Sustentable, Universidad Intercultural del Estado de Puebla, Huehuetla, Mexico*

Abstract

Mexico is the third producer of strawberry (*Fragaria ananassa* Duchesne) in the world after the USA and China. However, year after year the strawberry crops suffer from large economic losses due to the incidence of insect pests. It should be mentioned that one of the most harmful pests for strawberry and most crops is *Bemisia tabaci* (Gennadius). Insecticides have been the most used products in strawberry, however, due to irrational use, resistance has been detected to some chemical compounds in the populations of *B. tabaci*. Application of biological control agents is an alternative to reduce this problem. In this work the interaction of *Bacillus thuringiensis* strain GP139 and *Beauveria bassiana* strain HPI-019/14 was evaluated for the biological control of *B. tabaci* nymphs in strawberry crop under laboratory and greenhouse conditions. The interaction between *B. thuringiensis* and *B. bassiana* showed to have an antagonist effect and mortalities below 70% for laboratory conditions and 50% for greenhouse conditions at low and medium concentrations of conidia (1×10^1 and 1×10^6 conidia / mL) and maintaining the constant concentration of total protein 40 µg / mL. At higher concentrations of conidia (1×10^7 and 1×10^8 conidia / mL) and maintaining the constant concentration of total protein 40 µg / mL the mortality increase above 90% for both conditions (laboratory and greenhouse) also observing an additive effect. It showed considerable potential for the use of *B. thuringiensis* and *B. bassiana* as key elements of an integrated pest management multi-component system for *B. tabaci* management. Clearly, programs like this have a great potential also to reduce selection pressures and development of resistance.

Key words: interaction, entomopathogens, *Bacillus thuringiensis*, *Beauveria bassiana*, *Bemisia tabaci*, strawberry, greenhouse.

Introduction

Mexico is the third producer of strawberry (*Fragaria ananassa* Duchesne) in the world after the USA and China, obtaining in 2016 an amount of 398,287 tons, equivalent to an increase in the national strawberry crop of 1.7%. The estimated value of the national strawberry production is 5,779 million pesos and it has a strong growth in areas of different localities of the states of Puebla, Mexico (Rodríguez-Ramírez *et al*., 2017).

However, strawberry crops have economic losses due to the incidence of insect pests (Gallardo-Granados *et al*., 2016). One of the most harmful pests for strawberry is *Bemisia tabaci* (Gennadius). This whitefly can reduce the yield of the crop directly when feeding on the tissue of the leaves, sucking the sap to the plant, stopping its growth and decreasing the amount of sugar in the fruit. It should also be mentioned that they indirectly produce a sticky honeydew that they excrete during their feeding, they can cover the leaves and sustain the growth of sooty mould (*Capnodium* spp.). The nymphs and adults of *B. tabaci* are also an important vector of viruses - strawberry mottle virus (SMoV), the strawberry wrinkling virus (SCV), strawberry palidosis virus (SPaV), the strawberry necrotic shock virus (SNSV), the strawberry marginally yellowing thin virus (SMYEV) and the strawberry latent ring necrotic virus - (Contreras *et al*., 2014).

For the control of *B. tabaci*, insecticides have been the most used products, however, due to irrational use and poor use, resistance has been detected to some chemical compounds in *B. tabaci* populations in the field in recent years (Feng *et al*., 2010). Therefore, it is essential to look for management strategies that replace these compounds and reduce environmental pollution (Lacey *et al*., 2015; Glare and O'Callaghan, 2017; Arthurs and Dara, 2018).

An alternative is the use of biological control agents, which leads to the implementation of various enemies organisms of pests and ultimately one of the fields of biological control that is gaining importance is the implementation of entomopathogenic microorganisms (Rusch *et al*., 2010). Precisely a little explored topic of biological control, but with a very promising future, is the interaction of entomopathogenic bacteria and fungi, however, there are few reports regarding the interactions between these microorganisms (Roy and Pell, 2000).

In this sense, this work aims to determine what type of interaction is carried out between *Bacillus thuringiensis* strain GP139 and *Beauveria bassiana* strain HPI-019/14 for the control of *B. tabaci* in strawberry crops.

Table 1. Origin of the strains of *B. thuringiensis* and *B. bassiana* used in the investigation.

Strain	Origin of the strain	Order - Family
B. thuringiensis GP139	*Bemisia tabaci*	Hemiptera - Aleyrodidae
B. bassiana HPI-019/14	*Melanaphis sacchari*	Hemiptera - Aphididae

In relation to *B. thuringiensis*, it is a novel alternative in the control of *B. tabac*i because there are few reports on the implementation of this microorganism in the biological control of this insect pest (Davidson *et al*., 1996, Al-Shayji and Shaheen, 2008, Salazar-Magallón *et al*., 2015). As described, *B. thuringiensis* produces various groups of active proteins, such as Cry and Cyt. In addition to Cry proteins, *B. thuringiensis* produces a number of extracellular compounds, including Vip, Parasporin and S-layer proteins (SLP), that contribute to virulence (Peña *et al*., 2006; Lozano *et al*., 2011; Salazar-Magallón *et al*., 2015). Recently, some studies have shown that SLP to be a new group of parasporal inclusions of *B. thuringiensis* (Zhu and Yu, 2008; Guo *et al*., 2008; Zhou *et al*., 2011).

Strain GP139 of *B. thuringiensis* was isolated from a corpse of *B. tabaci* and previous reports have shown pathogenicity up to 94% mortality against nymphs and adult of *B. tabaci*, the strain was also characterized using PCR analysis and was shown to have the following genes: *cry*1Aa, *cry*1Ab, *cry*1Ac, *cry*1B, *cry*1D, *cry*2 and *s-layer gp*1, and later proved to be responsible for having the toxic activity on *B. tabaci* (Peña and Bravo, 2002; Peña *et al*., 2006; Salazar-Magallón *et al*., 2015).

Similar SLP highly similar to previously described SLP in *Bacillus anthracis* (EA1) and *Bacillus licheniformis* (OlpA), the phylogenetic relationships among SLP from different bacteria showed that these proteins from *Bacillus cereus*, *Bacillus sphaericus*, *B. anthracis*, *B. licheniformis*, and *B. thuringiensis* are arranged in the same main group, suggesting similar origins (Mesnage *et al*., 2001). However, the mechanism of action of this SLP is unknown, but we think that similar SLP creates an antigen-antibody reaction decreasing the insect's immunological defences, giving rise to individuals susceptible to the attack of a second entomopathogen (Allievi *et al*., 2014).

Concerning the entomopathogenic fungus *B. bassiana*, its use is common and its formulations are those of greater commercialization and use as biological control of pests, strain HPI-019/14 was isolated from the yellow sugarcane aphid, *Melanaphis sacchari* (Zehntner). Laboratory and field studies have revealed that *B. bassiana* is an excellent pathogen of *B. tabaci* and *Trialeurodes vaporariorum* (Westwood) when applied directly as a concentrated conidial suspension (Quesada-Moraga *et al*., 2006). Moreover, this fungus, when isolated from an insect of the same family as *B. tabaci*, could have a greater pathogenicity when associated with *B. thuringiensis* (Quesada-Moraga *et al*., 2006).

However, acceptance of biological products has been limited, probably due to the general perception among farmers that, compared to conventional chemicals, they are not fast acting and lose their effectiveness quickly (Rusch *et al*., 2010), but it could be possible that new findings on pathogenic *Beauveria* strains will be applied (Bedini *et al*., 2016).

Therefore, to control both nymphs and adults of *B. tabaci* more efficiently, it is necessary to select microorganisms that combine their best characteristics as a high pathogenicity against target organisms (Ibarra *et al*., 2006; Badii *et al*., 2006). Therefore, the aim of this work is to evaluate the interaction of a bacterium *B. thuringiensis* strain GP139 and the fungus *B. bassiana* strain HPI-019/14 for the biological control of *B. tabaci* nymphs in strawberry cultures under laboratory and greenhouse conditions.

Materials and methods

Microorganisms

The microorganisms used (table 1) in this investigation correspond to the bacterium *B. thuringiensis* strain GP139, belonging to the collection of the Plant Parasitology Laboratory of the Biological Research Center of Morelos State Autonomous University and to the strain of the fungus *B. bassiana* HPI-019/14 belonging to the collection of the Biological Control Laboratory of the Biotechnology Research Center of the Morelos State Autonomous University.

Cultivation of *B. bassiana* and production of conidia

The *B. bassiana* strain HPI-019/14 was cultured in an SDA medium containing: meat peptone (5 g/L), casein peptone (5 g/L), dextrose (40 g/L) and agar (15 g/L), the pH adjusted to a value of 5.6 ± 0.2 and cultures were incubated in the dark at a temperature of 30 °C for 15 days until complete sporulation and were subsequently stored at a temperature of 4 °C. The culture medium implementing for *B. bassiana* HPI-019/14 at the time of activation was PDA containing: infusion of potato (4 g/L), dextrose (20 g/L) and agar (20 g/L) plus Yeast Extract (2.5 g/L), incubated at 30 °C for 15 days until complete sporulation (Murillo-Alonso *et al*., 2015). For the sporulation stage, an alternative medium based on 120 g of sweet potato was implemented, which was boiled in 500 mL of distilled water, the mixture was homogenized with a blender, 7.5 grams of agar were added and the pH was adjusted to 7.0, then a sample of the activated strain was taken and cultivated in Petri dishes for 15 days at 30 °C until full sporulation. Subsequently, the conidia were collected from the Petri dishes using distilled water with Tween 20 at 1% v/v with a sterile handle. All this process was carried out in sterile conditions. After obtaining the aerial conidia, they were counted in a Neubauer chamber. Later the concentrations were adjusted to the level needed for treatments.

Cultivation of *B. thuringiensis* and obtaining the spore-crystal complex

The GP139 strain of *B. thuringiensis* was preserved in strips of sterile Whatman filter paper and kept in sterile Eppendorf tubes at room temperature (Salazar-Magallón *et al*., 2015), subsequently, the strain was activated in LB medium for 72 h until obtaining 90% of sporulation, then the strain was cultivated in solid SP medium which contains: nutritive broth (8 g/L), MgSO4 7H2O (0.250 g/L), KCl (1 g/L), MnCl2 4H2O (1.98×10^{-3} g/L), Fe2SO4 H2O (0.0732 g/L), H2 SO4 (3.2×10^{-3} g/L), CaCl2 (0.196 g/L) and agar (15 g/L), the pH was adjusted to a value of 7.0 ± 0.2. Once the culture sporulated completely (Salazar-Magallón *et al*., 2015) (checked under a microscope) the spore-crystal complex was recovered in sterile distilled water (one box in 5 ml of sterile water). The total protein concentration was quantified by the Bradford technique (Bradford, 1976), adjusting the concentrations for the foliar spray bioassay.

Pathogenicity test on nymphs of *B. tabaci* in laboratory

The pathogenicity of strain GP139 of *B. thuringiensis* and *B. bassiana* strain HPI-019/14 was evaluated under laboratory conditions (temperature, RH, light/dark) against nymphs of *B. tabaci* reared in young strawberry plants kept buckets with anti-aphid mesh in the Bioprocesses laboratory of Puebla State Popular Autonomous University. Plants infested with 3rd and 4th instar nymphs were sprinkled with an airbrush of compressed air according to the following treatments: a spores-crystals suspension of *B. thuringiensis* strain GP139 at a concentration of (0.04 mg/mL) of total protein (Guo *et al*., 2008; Zhou *et al*., 2011; Salazar-Magallón *et al*., 2015), a suspension of conidia of *B. bassiana* HPI-019/14 at a concentration of 1×10^6 conidia/mL (Adames-Mancebo *et al*., 2010), a suspension of spore-crystals strain GP139 *B. thuringiensis* and conidia of *B. bassiana* HPI-019/14 (0.04 mg/mL and 1×10^6 conidia/mL) respectively and water plus dispersant (Dimethyl sulfosuccinate 1 mL/L) as control. Each treatment (20 mL) was applied at distance of 20 cm for 1 minute (Salazar-Magallón *et al*., 2015). Twenty nymphs of 3rd and 4th instar of *B. tabaci* per strawberry plant. The experimental unit consisted of a strawberry plant. Three repetitions (three different plants) were performed for each treatment.

Bioassay of interaction between *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 against nymphs of *B. tabaci* under greenhouse conditions

An evaluation was made to determine the compatibility between *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 under greenhouse conditions in a commercial strawberry crop for the control of nymphs of *B. tabaci*. Plants infested with nymphs of 3rd and 4th instar were sprayed with an airbrush of compressed air according to the following treatments: 8 different concentrations of conidia (H1 - H8) were evaluated separately from *B. bassiana* HPI-019/14 (1×10^1 - 1×10^8 conidia/mL), the spore-crystal complex was also evaluated separately from *B. thuringiensis* GP139 at a concentration of 0.04 mg/mL of total protein (B1), and the interaction of 8 different concentrations of conidia of *B. bassiana* HPI-019/14 (1×10^1 - 1×10^8 conidia/mL) keeping the spore-crystal mixture of *B. thuringiensis* GP139 constant at a concentration of 0.04 mg/mL of total protein (A1 - A8) and finally, dispersant plus water (Dimethyl sulfosuccinate 1 mL/L) was used as a control. Each treatment (100 mL) was applied at distance of 20 cm for 1 minute (Salazar-Magallón *et al*., 2015). Twenty nymphs of 3rd and 4th instar of *B. tabaci* per strawberry plant were tested. The experimental unit consisted of a strawberry plant. Three repetitions (three different plants) were performed for each treatment.

Determination of interactive effects between entomopathogens

To determine the effects of the interaction between *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 the observed mortalities were compared with the expected mortalities under the assumptions of an independent effect (Mwamburi *et al*., 2009). The expected mortality (Bt-BbE) for the interaction *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 was calculated as follows:

$$\text{Bt-BbE} = \text{MBt} + \text{MBb} (1 - \text{MBt}),$$

Where MBt and MBb are the proportional mortalities caused by *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 alone respectively. The results of a calculated χ^2 test were compared to the table values of χ^2 (df = 1, $P \leq 0.05$):

$$\chi^2 = (O - E)\, 2 / E,$$

where O is the observed mortality for any treatments in the interaction (A1 - A8) and E is the expected mortality for any of the MBt-MBbE calculated concentrations. An additive effect would be indicated if $\chi^2 < 3.84$. On the other hand, if $\chi^2 > 3.84$ (Mwamburi *et al*., 2009), there would be reasons to suspect that the interaction was not additive (i.e., synergistic or antagonistic effect between the 2 agents). If O <E, the interaction will be considered antagonistic. The synergy would be indicated if O >E.

Statistical analysis

A completely randomized design was used for the study factors. For those variables that presented significant differences between their treatments, a mean comparison was made by the Tukey's method at a significance level of 0.05.

Results

The effects of the application of entomopathogenic organisms for the control of *B. tabaci* in laboratory conditions and concentrations recommended by authors and suppliers are presented in table 2, where the results of the individual evaluation of *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 are shown, in addition to the combination of both. The nymphs infected with *B. thuringiensis* GP139 showed an increase in the movement of their spiracles, as well as of their anal pores, abnormalities in their form were also observed. Once dead, the nymphs of *B. tabaci* showed a liquefaction and later a rupture of the cuticle to release the degraded fluid of

Table 2. Effects of foliar spraying of *B. bassiana* HPI-019/14, *B. thuringiensis* GP139 and the interaction at constant concentration against nymphs of *B. tabaci* in strawberry plants under laboratory conditions.

Treatment	Mortality in laboratory (%)
B. bassiana HPI-019/14 (1×10^6 conidia/mL)	61.67 ± 1.67 b
B. thuringiensis GP139 (0.04 mg/mL)*	71.67 ± 1.67 a
Interaction between *B. bassiana* HPI-019/14 and *B. thuringiensis* GP139	61.67 ± 6.01 b
Control (water + dispersant)	6.67 ± 1.67 c

The average of the foliar spray is presented at 7 days of treatment ± standard error, treatments with different letters indicate significant differences through a Tukey test (α 0.05). *total protein concentration. The number of nymphs used was 20 for each repetition.

the nymphs, as well as a yellow-brown coloration. Seven days after the spraying with treatment of *B. thuringiensis* GP139, a mortality of 71.67% at a concentration of 0.04 mg/mL of total protein could be observed, finding significant differences with respect to the other treatments (F = 66.05; P <0.001), on the other hand, when *B. bassiana* HPI-019/14 was evaluated, a lower mortality was observed (61.67%) at a concentration of 1×10^6 conidia/mL, at the same time, when the combination of both was evaluated. The same mortality was observed (61.67%), there being no significant differences between these treatments.

However, under the conditions mentioned above, no conclusive evidence was found that the interaction of *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 contributed to a better control of *B. tabaci.* Therefore, data of different concentrations of conidia of *B. bassiana* HPI-019/14, and one treatment with constant the protein concentration of *B. thuringiensis* GP139, and the interaction of *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 at different concentration in both laboratory and greenhouse conditions are shown in figure 1.

All the treatments of *B. bassiana* HPI-019/14 evaluated individually, both in laboratory and greenhouse conditions showed significant differences with respect to the control on the mortality of nymphs of *B. tabaci* (F = 22.0, P <0.001; F = 39.72, P <0.001 respectively). The nymphs killed by the fungus quickly dehydrated and remained attached to the leaf. Also, the nymphs infected by *B. bassiana* HPI-019/14 clearly showed a red to red brown colour. In the H1-H5 treatments (1×10^1 - 1×10^5 conidia/mL), mortalities below 50% were observed both in the laboratory and in the greenhouse, which had no significant differences between them (F = 6.36, P = 0.08; F = 15.3, P <0.001 respectively), on the other hand, when the concentration of conidia (H6 treatment) increased both in the laboratory and in the greenhouse, the percentage of mortality was 56.67% for

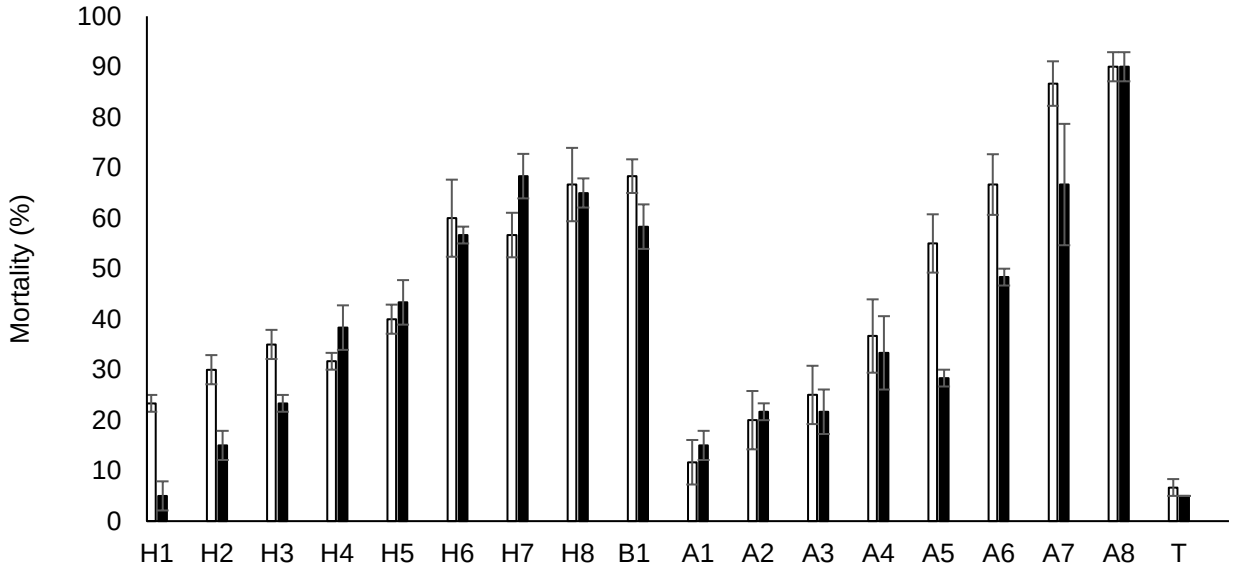

Figure 1. Effects of foliar spray of *B. bassiana* HPI-019/14, *B. thuringiensis* GP139 and the interaction of entomopathogens against *B. tabaci* nymphs in strawberry plants under laboratory and greenhouse conditions. The average of the foliar spray is presented at 7 days of treatment ± the standard error. The number of nymphs used was 20 for each repetition. H = *B. bassiana* treatments (conidia/mL), H_1: 1×10^1, H_2: 1×10^2, H_3: 1×10^3, H_4: 1×10^4, H_5: 1×10^5, H_6: 1×10^6, H_7: 1×10^7 and H_8: 1×10^8. B = *B. thuringiensis* treatments, B_1: 0.04 mg/mL. A = interactions treatments (conidia/mL-mg/mL), A_1: 1×10^1 - 0.04, A_2: 1×10^2 -0.04, A_3: 1×10^3 - 0.04, A_4: 1×10^4 - 0.04, A_5: 1×10^5 - 0.04, A_6: 1×10^6 - 0.04, A_7: 1×10^7 - 0.04 and A_8: 1×10^8 - 0.04. T = control treatment. White bars = laboratory conditions. Black bars = greenhouse conditions.

Table 3. Effects of the interaction between *B. bassiana* HPI-019/14 and *B. thuringiensis* GP139 against nymphs of *B. tabaci* through foliar spray in laboratory and greenhouse conditions.

Treatment	Test in laboratory				Test in greenhouse			
	Mortality (%)		χ^2	Interaction degree	Mortality (%)		χ^2	Interaction degree
	(E) Expected	(O) Observed			(E) Expected	(O) Observed		
A1	75.72	11.67	54.17	Antagonist	69.91	15.00	43.13	Antagonist
A2	77.83	20.00	42.97	Antagonist	73.08	21.67	36.17	Antagonist
A3	79.41	25.00	37.28	Antagonist	75.72	21.67	38.58	Antagonist
A4	78.36	36.67	22.18	Antagonist	80.47	28.33	33.78	Antagonist
A5	81.00	55.00	8.34	Antagonist	82.05	33.33	28.93	Antagonist
A6	87.33	66.67	4.89	Antagonist	86.28	48.33	16.69	Antagonist
A7	86.28	86.67	0.002	Additive	89.97	67.67	6.03	Antagonist
A8	89.44	93.33	0.169	Additive	88.92	90.00	0.01	Additive

both cases, there being significant differences with respect to the previous treatments ($F = 10.59$, $P < 0.001$; 23.08, $P < 0.001$ respectively), moreover, in the H7 treatment, a mortality percentage of 60.00% was observed for laboratory conditions, being statistically similar to the previous data but different from the rest of the treatments ($F = 12.09$, $P < 0.001$) and mortality of 68.33% for the greenhouse conditions, there were significant differences between the previous treatments ($F = 27.39$, $P < 0.001$), however, in the H8 treatment under laboratory conditions an increase in mortality of 67.67% was observed, being statistically different from the previous treatments ($F = 12.17$, $P < 0.001$), however under greenhouse conditions the mortality percentage remained the same at 68.33% with respect to the previous treatment ($F = 30.96$, $P < 0.001$). The mortality related to *B. bassiana* HPI-019/14 increased as the concentration of conidia increased.

On the other hand, when the bacterium *B. thuringiensis* GP139 was evaluated at a concentration of 0.04 mg/mL of total protein, a mortality of 68.33% was observed in laboratory conditions, finding statistical differences with respect to control and fungal treatments ($F = 25.05$, $P < 0001$), however under greenhouse conditions, on average mortality was kept low 58.33%, during the course of the test, finding significant differences between fungal treatments ($F = 38.33$, $P < 0.001$), while average mortality in treatments with fungi (H6 - H8) exceed this mortality.

The effects of the application of *B. thuringiensis* GP139 in interaction with *B. bassiana* HPI-019/14 under laboratory and greenhouse conditions are shown in table 3. The mortalities caused by the interaction of these two entomopathogenic agents had significant differences both in the laboratory and greenhouse conditions ($F = 22.47$, $P < 0.001$; $F = 14.17$, $P < 0.001$) respectively. At low concentrations of conidia (treatments A1 - A4) mortalities were observed below 40% in laboratory conditions ($F = 5.35$, $P = 0.014$) and below 30% in greenhouse ($F = 9.02$, $P = 0.002$), there being no significant differences between the treatments, likewise an antagonistic effect was observed at these concentrations. When the A5 treatment was evaluated, an increase in mortality of 55.00% was observed under laboratory conditions, this value is statistically different from the previous treatments ($F = 10.58$, $P < 0.001$, $\chi^2 = 8.34$, $P < 0.05$, $df = 1$), however, in the greenhouse evaluation mortality remains well below the expected with a value of 33.33% ($F = 9.69$, $P = 0.001$, $\chi^2 = 28.93$, $P < 0.05$, $df = 1$), the nymphs exposed to this interaction they showed an antagonistic effect. When the A6 treatment was evaluated although there is an increase in the percentage of mortality under laboratory conditions 66.67%, statistically there are no significant differences with respect to the previous treatment ($F = 15.79$, $P < 0.001$, $\chi^2 = 4.89$, $P < 0.05$, $df = 1$), however, under the greenhouse the observed mortality of 48.33% statistically has no significant difference with respect to the previous treatment ($F = 17.30$, $P < 0.001$, $\chi^2 = 16.69$, $P < 0.05$, $df = 1$), the nymphs exposed to these conditions showed an antagonistic effect. However, when the concentration of conidia was increased, treatment A7, an increase in mortality was observed, in the laboratory a value of 86.67% was recorded ($F = 25.00$, $P < 0.001$, $\chi^2 = 0.002$, $P < 0.05$, $df = 1$), an additive effect was observed under these test conditions, while in the greenhouse a mortality of 67.67% was observed ($F = 10.72$, $P < 0.001$, $\chi^2 = 6.03$, $P < 0.05$, $df = 1$) in the greenhouse the interaction of these entomopathogens showed an antagonistic effect, these values have significant differences with respect to the treatments previously mentioned. Finally, when the A8 treatment was evaluated, a significant increase was observed in the 93.33% mortality percentage, although statistically there is no significant difference with the previous treatment ($F = 22.47$, $P < 0.001$, $\chi^2 = 0.169$, $P < 0.05$, $df = 1$) in the same way as the previous treatment, the interaction showed an additive effect, however, under greenhouse conditions an increase in mortality of 90.00% was observed ($F = 14.17$, $P < 0.001$, $\chi^2 = 0.01$, $P < 0.05$, $df = 1$), there being significant differences with the previous treatment and showing an additive effect when they interact at these concentrations.

Discussion

B. thuringiensis is a novel alternative in the control of nymphs of *B. tabaci* because there are few reports on the use of this entomopathogenic bacterium on this in-

sect pest (Davidson *et al*., 1996, Al-Shayji and Shaheen, 2008; Salazar-Magallón *et al*., 2015). The protein responsible for the toxic activity *B. thuringiensis* GP139 on nymphs of *B. tabaci* is an S-layer protein (Davidson *et al*., 1996, Salazar-Magallón *et al*., 2015), this protein is contained in a parasporal inclusion produced during its sporulation phase (Guo *et al*., 2008; Zhou *et al*., 2011). S-layers protein are now recognized as one of the most common envelope surface structures in Archaea and Bacteria. Moreover, detailed studies on structure, chemical composition, genetics, morphogenesis, surface and permeability properties revealed that S-layers are the simplest biological membranes developed during evolution and according to recent studies it seems that S-layer proteins are present in some varieties of *B. thuringiensis* (Sleytr, 1997; Sleytr and Beveridge, 1999; Mesnage *et al*., 2001; Wang *et al*., 2004; Gou *et al*., 2008; Zhou *et al*., 2011; Dong *et al*., 2015). Nevertheless, S-layer proteins have been associated with pathogenesis in some species of the genus Bacillaceae, that is, the S-layer protein of *B. thuringiensis* GP1 is toxic for the Coleoptera *Epilachna varivestis* Mulsant (Peña *et al*., 2006), the protein A (S-layer) of *B. anthracis* mediates the adherence of vegetative cells to human cells (Kern and Schneewind, 2008), the S-layer protein of *Lysinibacillus sphaericus* showed to have larvicidal activity against the mosquito *Culex quinquefasciatus* Say (Lozano *et al*., 2011) and finally the S-layer protein of *B. thuringiensis* strain GP139 showed to have toxic activity against nymphs of *B. tabaci* (Salazar-Magallón *et al*., 2015). It would be relevant to test whether other S-layer proteins produced by *B. thuringiensis* have some insecticidal activity against other insect species. This knowledge could be important for future control of insects. However, the mechanism of action of this SLP is unknown, and future work is needed to describe it. However, it has been shown that some SLP have the function of being antigenic determinants or epitopes and generate an antibody antigen reaction, lowering the host's immune system and rendering it susceptible to attack by an opportunist or a second pathogen (Engelhardt, 2007).

When *B. thuringiensis* GP139 was applied as the only treatment, mortalities of 68.33% and 58.33% were obtained under laboratory and greenhouse conditions respectively. These results agree with that reported by Al-Shayji and Shaheen (2008), who mention mortalities of 50 to 60% in first instar nymphs of *B. tabaci* treated with a strain of *B. thuringiensis* (isolated in Kuwait and grown in a medium of commercial culture) at a concentration 12.5 times (500 µg/mL) higher than ours (40 µg/mL). However, previous studies showed that *B. thuringiensis* GP139 reaches mortalities at a concentration of 40 µg/mL greater than 80% 2 days after treatment to more than 90% after 8 days, however, in this study strain GP139 was cultivated in alternative media made with industrial by-products, suggesting an increase in toxicity when grown in these alternative media.

The control of nymphs applying *B. bassiana* HPI-019/14 as the only treatment was not our objective. However, the poor performance of treatments with *B. bassiana* was unexpected. Previous research with *B. bassiana* against nymphs of *B. tabaci* have shown mortalities up to 90% compared to 67.67% reported in this work both in laboratory and greenhouse conditions 7 days after application (Vicentini *et al*., 2001, Quesada-Moraga *et al*., 2006, Santiago-Álvarez *et al*., 2006, Torrado-León *et al*., 2006). In our work, the mortality levels of *B. bassiana* HPI-019/14 on nymphs of *B. tabaci* were similar to those caused by *Aschersonia* spp. (Meekes *et al*., 2002), but less than the levels reported between 70 and 95% of mortality caused by other fungi such as *Metarhizium anisopliae*, *Paecylomices fumosoroseus* and *Verticillium lecanii* (Wang *et al*., 2007; Taylor and Khan, 2010). This comparison, must be interpreted with care, since the fungal isolations used in the last studies were passed through the whitefly before experimentation, while our isolates did not pass through a host. For most entomopathogenic fungi consecutive passages through the host increases virulence and repeated subculture in an artificial environment can decrease it (Brownbridge *et al*., 2001; Quesada-Moraga and Vey, 2003).

The potential of the strains to be improved biotechnologically to kill the insect host faster. In this sense, the interaction of *B. thuringiensis* GP139 was proposed as a first infection with the entomopathogenic fungus *B. bassiana* HPI-019/14 as a second infection for the control of nymphs of *B. tabaci* in strawberry crops under greenhouse conditions. The addition of the *B. bassiana* HPI-019/14 sprays to treatments with *B. thuringiensis* GP139 offered some advantage to both agents in terms of mortality and inhibition of the emergence of nymphs to adults. However, there was evidence of antagonistic and additive interaction between *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14. At low and medium concentrations of conidia (A1 - A6) mortalities were observed below 70% in both laboratory and greenhouse conditions, although 30 to 60% of live nymphs showed signs of fungal development and severe dehydration caused by the infection of *B. thuringiensis* GP139. These results agree with what reported by Mwanburi *et al*., 2009, they report that the interaction at low doses of *B. bassiana* conidia and a constant concentration of *B. thuringiensis* the effect of mortality on common fruit fly larvae are decreased by up to 70-80% because it is possible that the balance of nutrients is affected by the action of the fungus and bacteria during the initial processes of infection. An immune response is expected in nymphs that survive treatment with the entomopathogen. Although insects do not possess mechanisms to produce antibodies as such, they present cellular defence reactions such as phagocytosis, nodulation and encapsulation, as well as proteolytic cascades among others, as part of their humoral defence capacity (Strand, 2008). Unfortunately, although insect cell defence reactions against fungi and bacteria are well documented, the humoral mechanisms of immunity have not been convincingly investigated in insects (Lavine and Strand, 2002; Ishii *et al*., 2010). The potential of the interaction between *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 could increase if the nymphs were fed with the pure protein of the bacteria in the first stage, our hypothesis is that the protein

S-layer is introduced to the body of the nymphs through some natural aperture as spiracles or anal orifice, because *B. tabaci* apparently does not possess digestive proteases as demonstrated both by biochemical assays and the failure of a trypsin/chymotrypsin inhibitor to induce mortality or reduce honeydew production. For this reason the *B. thuringiensis* delta-endotoxins were fed both before and after trypsin-activation and later spraying conidia of the fungus, this process triggers a physiological stress of the nymph causing it to be more susceptible to attack by a second entomopathogen (Costa *et al*., 2001; Xiao-Mu *et al*., 2008; Meissle *et al*., 2009).

When the concentration of conidia was increased (treatments A7 and A8) the mortality increased considerably reaching values between 80 and 90% in laboratory conditions, also finding evidence of an additive effect between the entomopathogenic microorganisms, but under greenhouse conditions with the A7 treatment mortality is below 70% and in the A8 treatment mortality reached 90%. Also under these conditions it was observed that both live and dead nymphs had a dry reddish coloration as a probable consequence of the production of secondary metabolites, furthermore, after 7 days retention or interference in the process of moulting was observed. The presence of mycelium or fungal spores in the exuvia stored in humid chambers suggest that this phenomenon is a sublethal effect attributable to the application of *B. bassiana*. Considering that the moulting process depends on the nutrients for the formation of the new cuticle (Murillo-Alonso *et al*., 2015), several authors have shown that toxic metabolites play an important role in the reduction of proteins, amino acids and nucleic acids (Molnar *et al*., 2010; Gibson *et al*., 2014). In support of this, Davidson *et al*., 1996, recorded alterations in the adult physiology of *Bemisia argentifolii* Bellows et Perring by exposing them to food treated with partially purified destruxin of *M. anisopliae* at concentrations of 18.0, 3.0 and 1.8 µg/ml. They noted a substantial reduction in the excretion of honeydew for all three concentrations before death occurred.

Our findings of an additive interaction between *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 contrast with the results obtained by several authors, the reasons for this are not known; however, any comparative interpretation must consider the many variables. There are no reports evaluating the interaction of entomopathogens on nymphs of *B. tabaci*, however, our results agree with the data obtained in a study by Costa *et al*. (2001) in which no synergy was observed if not an additive effect between *B. thuringiensis tenebrionis* and *B. bassiana* strain GHA applied against *Leptinotarsa decemlineata* (Say). These authors did not apply the entomopathogens simultaneously, in addition, they applied the pure toxin (CryIIIA), the endotoxin was applied against early fourth instar larvae, and the fungus was applied several days later against the fourth fully developed larvae (prepupae) that survived the poisoning. Also, our data agree with that reported by Mwamburi *et al*., 2009, where they found additive effects in the combination of a strain of *B. bassiana* and two strains of *B. thuringiensis*, the pathogens were applied in pure form or in formulations. However, our results do not match what was reported by Wraight and Ramos (2005), they report evidence of synergism between *B. thuringiensis* and *B. bassiana*. In view of this and taking into account the well documented potential for various interactions between biopesticides, we do not consider our tendency to synergism to be particularly surprising. The synergistic interaction may have resulted from the direct effects of the nymph poisoning on the rates of successful fungal penetration. It is well known that the moulting process can eliminate the infectious fungal inoculum of an insect and thus prevent the host from infection (Avery *et al*., 2010). The hypothesis follows and therefore, *B. thuringiensis* could synergize with *B. bassiana* by prolonging the activity in time intervals between moulting (providing the fungus with more time to break the cuticle before being cast). A synergistic response would result if the physiology of the cuticle or haemolymph of the intoxicated hosts is not altered in any way by conferring resistance to penetration or colonization of fungi. This mode of action could explain the low and unpredictable level of synergy observed here. *B. bassiana* is highly pathogenic for yellowish aphid and the process of germination of the spore and infection is faster in favourable environmental conditions (Maketon *et al*., 2013).

Conclusions

No evidence was found of a synergistic effect between *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14, however, the additive effect observed both in the laboratory and in the greenhouse may be an indication of testing new conditions and concentrations of entomopathogenic organisms and increase the potential in the interaction of *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 for the control of *B. tabaci* in strawberry crops. In essence, the *B. thuringiensis* component would provide rapid control and foliage protection, while the *B. bassiana* component would contribute to the long-term suppression of subsequent generations (through persistent activity against nymphs, puparia, and adults). In addition, it demonstrated considerable potential for the use of *B. thuringiensis* GP139 and *B. bassiana* HPI-019/14 as key elements for *B. tabaci* integrated pest management.

Acknowledgements

We are grateful for the support of Concepción Carreón Cruz, for the support provided in the Biotechnology and Environmental Research Laboratory of the Popular Autonomous University of the State of Puebla. We also appreciate the support of the producers of Strawberry from the municipality of Zacatlán, Puebla Mexico, for lending their greenhouses for the realization of this experiment.

References

ADAMES-MANCEBO M., FERNÁNDEZ-RUVALCABA M., PEÑA-CHORA G., HERNÁNDEZ-VELÁZQUEZ V. M., 2010.- Effects of passages through a suitable host of the fungus, *Metarhizium anisopliae*, on the virulence of acaricide-susceptible and resistant strains of the tick, *Rhipicephalus Microplus*.- *Journal of Insect Science*, 11 (21): 1-13.

AL-SHAYJI Y., SHAHEEN N., 2008.- Isolation of *Bacillus thuringiensis* strain from Kuwait's soil effective against whitefly nymphs.- *Journal of Insect Science*, 8 (4): 4.

ALLIEVI M. C., PALOMINO M. M., PRADO-ACOSTA M., LANATI L., RUZAL S. M., 2014.- Contribution of S-layer proteins to the mosquitocidal activity of *Lysinibacillus sphaericus*.- *PLoS ONE*, 9 (10): e111114.

AVERY P. B., QUEELEY G. L., FAULL J., SIMMONDS M. S. J., 2010.- Effect of photoperiod and host distribution on the horizontal transmission of *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) in greenhouse whitefly assessed using a novel model bioassay.- *Biocontrol Science and Technology*, 20 (10): 1097-1111.

ARTHURS S., DARA S. K., 2018.- Microbial biopesticides for invertebrate pests and their markets in the United States.- *Journal of Invertebrate Pathology*, in press: doi:10.1016/j.jip.2018.01.008.

BADII M. H., ABREU J. L., 2006.- Biological control a sustainable way of pest control.- *International Journal of Good Conscience*, 1 (1): 82-89.

BEDINI S., SARROCCO S., BARONCELLI R., VANNACCI G., CONTI B., 2018.- Pathogenic potential of *Beauveria pseudobassiana* as bioinsecticide in protein baits for the control of the medfly *Ceratitis capitata*.- *Bulletin of Insectology*, 71 (1): 31-38.

BRADFORD M., 1976.- A rapid and sensitive method for the quantitation of micrograms quantities of protein utilizing the principle of protein-dye binding.- *Analytical Biochemistry*, 72 (1-2): 248-254.

BROWNBRIDGE M., COSTA S., JARONSKI S. T., 2001.- Effects of in vitro passage of *Beauveria bassiana* on virulence to *Bemisia argentifolii*.- *Journal of Invertebrate Pathology*, 77: 280-283.

CONTRERAS C. A., SILVA L., GALLEGOS V., ORTIZ M. L., JOFRE A. E., 2014.- Incidence of mixed viral infections in a strawberry producing area in Guanajuato, Mexico.- *Revista Mexicana de Fitopatología*, 32 (1): 12-25.

COSTA S. D., BARBERCHECK M. E, KENNEDY G. G., 2001.- Mortality of Colorado potato beetle (*Leptinotarsa decemlineata*) after sublethal stress with the CryIIIA d-endotoxin of *Bacillus thuringiensis* and subsequent exposure to *Beauveria bassiana*.- *Journal of Invertebrate Pathology*, 77: 173-179.

DAVIDSON E. W., PATRON R. B. R., LACEY L. A., FRUTOS R., VEY A., HENDRIX D. L., 1996.- Activity of natural toxin against the silverleaf whitefly, *Bemisia argentifolii*, using a novel bioassay system.- *Entomologia Experimentalis et Applicata*, 79 (1): 25-32.

DONG Z., JUNHUA L., JINSHUI Z., CE G., PENG D., SUN M., 2015.- Complete genome sequence of *Bacillus thuringiensis* CTC, a typical strain with high production of S-layer proteins.- *Journal of Biotechnology*, 220: 100-101.

ENGELHARDT H., 2007.- Are S-layers exoskeletons? The basic function of protein surface layers revisited.- *Journal of Structural Biology*, 160: 115-124.

FENG Y., WU Q., WANG S., CHANG X., XIE W., XU B., ZHANG Y., 2010.- Cross-resistance study and biochemical mechanisms of thiamethoxam resistance in B-biotype *Bemisia tabaci* (Hemiptera: Aleyrodidae).- *Pest Managnament Science*, 66: 313-318.

GALLARDO-GRANADOS S., SALAZAR-SOLÍS E., SALAS-ARAIZA M. D., MARTÍNEZ-JAIME O. A., 2016.- Incidence of species of Hemiptera in strawberry under two cultivation systems in Irapuato, Guanajuato, México.- *Southwestern Entomologist*, 41 (2): 547-560.

GIBSON D. M., DONZELLI B. G. G., KRASNOFFA S. B., KEYHANI N. O., 2014.- Discovering the secondary metabolite potential encoded within entomopathogenic fungi.- *Natural Product Reports*, 31: 1287-1305.

GLARE T. R., O'CALLAGHAN M., 2017.- Microbial biopesticides for control of invertebrates: Progress from New Zealand.- *Journal of Invertebrate Pathology*, in press: doi:10.1016/j.jip.2017.11.014.

GUO G., ZHANG L., ZHOU Z., MA Q., LIU J., ZHU C., ZHU L., YU Z., SUN M., 2008.- A newgroup of parasporal inclusions encoded by the S-layer gene of *Bacillus thuringiensis*.- *Microbiology Letters*, 282: 1-7.

IBARRA J. E., DEL RINCÓN M. C., GALINDO E., PATIÑO M., SERRANO L., GARCÍA R., CARRILLO J. A., PEREYRA-ALFÉREZ B., ALCÁZAR-PIZAÑA A., LUNA-OLVERA H., GALÁN-WONG L. J., PARDO L., MUÑOZ-GARAY C., GÓMEZ I., SOBERÓN M., BRAVO A., 2006.- Los microorganismos en el control biológico de insectos y fitopátogenos.- *Revista Latinoamericana de Microbiología*, 48 (2): 113-120.

ISHII K., HAMAMOTO H., KAMIMURA M., NAKAMURA Y., NODA H., IMAMURA K., MITA K., SEKIMIZU K., 2010.- Insect cytokine paralytic eptide (PP) induces cellular and humoral immune responses in the silkworm *Bombyx mori*.- *The Journal of Biological Chemistry*, 285 (37): 28635-28642.

KERN J., SCHNEEWIND O., 2008.- BslA, a pXO1-encoded adhesin of *Bacillus anthracis*.- *Molecular and Microbiology*, 68: 504-515.

LAVINE M. D., STRAND M. R., 2002.- Insect hemocytes and their role in immunity.- *Insect Biochemistry and Molecular Biology*, 32: 1295-1309.

LACEY L. A., GRZYWACZ D., SHAPIRO-ILAN D. I., FRUTOS R., BROWNBRIDGE M., GOETTEL M. S., 2015.- Insect pathogens as biological control agents: back to the future.- *Journal of Invertebrate Pathology*, 123: 1-41.

LOZANO L. C., AYALA J. A., DUSSÁN J., 2011.- *Lysinibacillus sphaericus* S-layer protein toxicity against *Culex quinquefasciatus*.- *Biotechnology Letters*, 33: 2037-2041.

MAKETON M., CHAKANYA N., PREM-UDOMKIT K., MAKETON C., 2013.- Interaction between entomopathogenic fungi and some aphid species in Thailand.- *Gesunde Pflanzen*, 65: 93-105.

MEEKES E. T. M., FRANSEN J. J., VAN LENTEREN J. C., 2002.- Pathogenicity of *Aschersonia* spp. against whiteflies *Bemisia argentifolii* and *Trialeurodes vaporariorum*.- *Journal of Invertebrate Pathology*, 81: 1-11.

MEISSLE M., PILZ C., ROMEIS J., 2009.- Susceptibility of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to the entomopathogenic fungus *Metarhizium anisopliae* when feeding on *Bacillus thuringiensis* Cry3Bb1-Expressing maize.- *Applied and Environmental Microbiology*, 75 (12): 3937-3943.

MESNAGE S., HAUSTANT M., FOUET A., 2001.- A general strategy for identification of S-layer genes in the *Bacillus cereus* group: molecular characterization of such a gene in *Bacillus thuringiensis* subsp. *galleriae* NRRL 4045.- *Microbiology*, 147: 1343-1351.

MOLNAR I., GIBSON D. M., KRASNOFF S. B., 2010.- Secondary metabolites from entomopathogenic Hypocrealean fungi.- *Natural Product Reports*, 27: 1241-1275.

MURILLO-ALONSO K. T., PEÑA-CHORA G., HERNÁNDEZ-BALTAZAR E., HERNÁNDEZ-VELÁZQUEZ V. M., 2015.- Conidia production by *Isaria fumosorosea* on solid substrates and its pathogenicity towards *Bemisia tabaci*.- *Biocontrol Science and Technology*, 25 (2): 175-184.

MWAMBURI L. A., LAING M. D., MILLER R., 2009.- Interaction between *Beauveria bassiana* and *Bacillus thuringiensis* var. *israelensis* for the control of house fly larvae and adults in poultry houses.- *Poultry Science*, 88: 2307-2314.

PEÑA G., BRAVO A., 2002.- Discovery of a new *Bacillus thuringiensis* toxins, pp. 106-111. In: *Biotechnology of* Bacillus thuringiensis *and its environmental impact* (AKHURST R. J., BEARD C. E., HUGHES P., Eds).- Proceedings of the 4th Pacific Rim Conference, CSRO.

PEÑA G., MIRANDA-RIOS J., DE LA RIVA G., PARDO-LÓPEZ L., SOBERÓN M., BRAVO A., 2006.- A *Bacillus thuringiensis* S-layer protein involved in toxicity against *Epilachna varivestis* (Coleoptera: Coccinellidae).- *Applied and Environmental Microbiology*, 72 (1): 353-360.

QUESADA-MORAGA E., VEY A., 2003.- Intra-specific variation in virulence and in vitro production of macromolecular toxins active against locust among *Beauveria bassiana* strains, and effects of in vivo and in vitro passages on these factors.- *Biocontrol Science Technology*, 13: 323-340.

QUESADA-MORAGA E., MARANHAO E. A. A., VALVERDE-GARCÍA P., SANTIAGO-ÁLVAREZ C., 2006.- Selection of *Beauveria bassiana* isolates for control of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* on the basis of their virulence, thermal requirements, and toxicogenic activity.- *Biological Control*, 36: 274-287.

RODRÍGUEZ-RAMÍREZ F., GARCÍA-ORTEGA I., VASQUEZ-GAMBOA S. A., JUAREZ-MENDOZA L. 2017.- Análisis, diseño e implementación de un invernadero automatizado para la producción de fresa en Tehuacán.- *Revista del Desarrollo Tecnológico*, 1 (3): 8-17.

ROY H. E., PELL J. K., 2000.- Interactions between entomopathogenic fungi and other natural enemies: implications for biological control.- *Biocontrol Science and Technology*, 10: 737-752.

RUSCH A., VALANTIN-MORISON M., SARTHOU J. P., ROGER-ESTRADE J., 2010.- Biological control of insect pests in agroecosystems: effects of crop management, farming systems, and seminatural habitats at the landscape scale: a review.- *Advances in Agronomy*, 109: 219-259.

SALAZAR-MAGALLON J. A, HERNANDEZ-VELAZQUEZ V. M., ALVEAR-GARCIA A, ARENAS-SOSA I., PEÑA-CHORA G., 2015.- Evaluation of industrial by-products for the production of *Bacillus thuringiensis* strain GP139 and the pathogenicity when applied to *Bemisia tabaci* nymphs.- *Bulletin of Insectology*, 68 (1): 103-109.

SANTIAGO-ALVAREZ C., MARANHAO E. A., MARANHAO E., QUESADA-MORAGA E., 2006.- Host plant influences pathogenicity of *Beauveria bassiana* to *Bemisia tabaci* and its sporulation on cadavers.- *BioControl*, 51: 519-532.

SLEYTR U. B., 1997.- Basic and applied S-layer research: an overview.- *FEMS Microbiology Reviews*, 20: 5-12.

SLEYTR U. B., BEVERIDGE T. J., 1999.- Bacterial S-layer.- *Trends in Microbiology*, 7 (6): 253-260.

STRAND M. R., 2008.- The insect cellular immune response.- *Insect Science*, 15: 1-14.

TORRADO-LEÓN E., MONTOYA-LERMA J., VALENCIA-PIZO E., 2006.- Sublethal effects of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) on the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) under laboratory conditions.- *Mycopathologia*, 62: 411-419.

TAYLOR B. M., KHAN A., 2010.- Germination, radial growth and virulence of *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* on *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae).- *Pakistan Entomologist*, 32 (2): 148-154.

VICENTINI S., FARIA M., OLIVEIRA M. R. V., 2001.- Screening of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) isolates against nymphs of *Bemisia tabaci* (Genn.) biotype B (Hemiptera: Aleyrodidae) with description of a new bioassay method.- *Neotropical Entomology*, 30 (1): 97-103.

WANG L., SUN M., YU Z., 2004.- Capacity of *Bacillus thuringiensis* S-layer protein displaying polyhistidine peptides on the cell surface.- *Applied Biochemistry and Biotechnology*, 119: 133-143.

WANG L., HUANG J., YOU M., GUAN X., LIU B., 2007.- Toxicity and feeding deterrence of crude toxin extracts of *Lecanicillium* (*Verticillium*) *lecanii* (Hyphomycetes) against sweet potato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae).- *Pest Management Science*, 63: 381-387.

WRAIGHT S. P., RAMOS M. E., 2005.- Synergistic interaction between *Beauveria bassiana*- and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae.- *Journal of Invertebrate Pathology*, 90: 139-150.

XIAO-MU M., XIAO-XIA L., XIA N., BO Z., FEI H., XIU-MIN G., YUN-FENG T., QING-WEN Z., 2008.- Effects of *Bacillus thuringiensis* toxin Cry1Ac and *Beauveria bassiana* on Asiatic corn borer (Lepidoptera: Crambidae).- *Journal of Invertebrate Pathology*, 99 :123-128.

ZHOU Z., PENG D., ZHENG J., GUO G., TIAN L., YU Z., SUN M., 2011.- Two groups of S-layer proteins, SLP1s and SLP2s, in *Bacillus thuringiensis* co-exist in the S-layer and in parasporal inclusions.- *BMB reports*, 44 (5): 323-328.

ZHU C., YU Z., 2008.- The surface layer protein of *Bacillus thuringiensis* CTC forms unique intracellular parasporal inclusion body.- *Journal of Basic Microbiology*, 48: 302-307.

Authors' addresses: Jesús Antonio SALAZAR-MAGALLÓN (corresponding author, jesus.salazar@uiep.edu.mx), División de Procesos Naturales, Licenciatura en Desarrollo Sustentable, Universidad Intercultural del Estado de Puebla, Calle Principal a Lipuntahuaca S/N, Lipuntahuaca, Huehuetla, CP 73475 Puebla, México; Carlos Eduardo SOMOZA-VARGAS, Luis Daniel ORTEGA-MARTÍNEZ, Facultad de Biotecnología, Universidad Popular Autónoma del Estado de Puebla, Av. 21 Sur #1103, Barrio de Santiago, CP 72410 Puebla, México; Víctor Manuel HERNÁNDEZ-VELÁZQUEZ, Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, col. Chamilpa, Cuernavaca, Morelos, México; Guadalupe PEÑA-CHORA, Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, col. Chamilpa, Cuernavaca, Morelos, México; Gerardo TORRES-GARCÍA, Centro de Investigación en Alimentos y Desarrollo, Av. De la Salud s/n entre Av. Aguamilpa y Blvd Colosio, Tepic, Nayarit, México; Arturo HUERTA-DE LA PEÑA, Laboratorio de Control Biológico-Colegio de Posgradudos, Carr. Federal México-Puebla Km. 125.5, Santiago Momoxpan, Puebla, México.

Received January 9, 2018. Accepted June 22, 2018.