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Published on: 13 May 2014 - Apidologie (Springer Paris)

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Eva Alquisira-Ramírez, José Paredes-Gonzalez, Víctor Hernández-Velázquez, José Ramírez-Trujillo, Guadalupe Peña-Chora. In vitro susceptibility of Varroa destructor and Apis mellifera to native strains of Bacillus thuringiensis . Apidologie, Springer Verlag, 2014, 45 (6), pp.707-718. 10.1007/s13592-014-0288-z . hal-01234771

HAL Id: hal-01234771

https://hal.archives-ouvertes.fr/hal-01234771

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Apidologie (2014) 45:707–718
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DOI: 10.1007/s13592-014-0288-z

Original article

In vitro susceptibility of *Varroa destructor* and *Apis mellifera* to native strains of *Bacillus thuringiensis*

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Received 1 May 2013 – Revised 19 March 2014 – Accepted 10 April 2014

Abstract – Fifty-four *Bacillus*-like strains were isolated from dead *Varroa destructor* collected in 24 colonies of bees from seven apiaries. The total proteins of 22 isolates were evaluated against adult female *V. destructor* (100 μg/mL), but only nine caused over 80 % mortality. The LC_{50} of EA26.1, EA3 and EA11.3 isolates were calculated. The most virulent isolate was EA26.1, as 96.7 % of the mites were killed 36 h after treatment, and the LC_{50} was 1.50 μg/mL. EA3 produced 93.3 % mite mortality after 48 h treatment, with an LC_{50} of 7.1 μg/mL. Finally, EA11.3 produced 93.3 % mite mortality after 60 h treatment, with an LC_{50} of 22.8 μg/mL. Additionally, the EA3 and EA26.1 isolates were used to determine mortality on adults and larvae of worker bees of *A. mellifera*. When the 16S rRNA gene was amplified and sequenced, the three strains were found to belong to the species *B. thuringiensis*. The results showed that honey bee adults and larvae are not negatively affected by identified *B. thuringiensis* isolates, indicating that they could be successfully used for the biological control of *V. destructor* without adverse effects on adults and larvae of *A. mellifera*, and reduce the negative impact of this mite on colonies.

***Varroa destructor* / *Apis mellifera* / *Bacillus thuringiensis* / pathogenicity / virulence**

1. INTRODUCTION

Varroa destructor Anderson and Trueman (Acari: Varroidae) is an ectoparasitic mite that feeds on the haemolymph of bees *Apis mellifera* Linnaeus (Hymenoptera: Apidae) (Anderson and Trueman 2000). This mite is recognized as the most serious pest in beekeeping worldwide because it causes open wounds on the cuticle of the bees, weakening their immune system and allowing for the entry of microorganisms such as bacteria, fungi and viruses that cause severe damage to the bee population (Brødsgaard et al. 2000; Gliński and Jarosz 1992; Nordstrőm 2003).

To control this mite, beekeepers currently use the following miticides: acrinathrin, amitraz,

Electronic supplementary material The online version of this article (doi:10.1007/s13592-014-0288-z) contains supplementary material, which is available to authorized users.

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Manuscript editor: Peter Rosenkranz

bromopropylate, chlordimeform, cimiazol, coumaphos, fluvalinate and flumethrin (Milani 1999). However, *V. destructor* has developed resistance to amitraz, coumaphos, fluvalinate and flumethrin (Colin et al. 1997; Elzen et al. 2000; Spreafico et al. 2001Thompson et al. 2002).

Additionally, in the case of miticides, their residue in honey and other products with higher levels than those permitted by international standards cause problems and could induce side effects in bees (Desneux et al. 2007; Kochansky et al. 2001; Martel et al. 2007; Wallner 1999).

Another way to control *V. destructor* is to use organic products such as oxalic acid, formic acid and essential oils. However, their use has not been well accepted by many beekeepers because they are difficult to apply, their effectiveness depends on the ambient temperature, and the sudden release of certain products may affect the bee brood and even cause death of bees (Bogdanov et al. 2002; Gregorc et al. 2004; Milani 2001; Desneux et al. 2007).

An alternative strategy to control *V. destructor* is the use of entomopathogenic microorganisms such as bacteria and fungi (Hamiduzzaman et al. 2012; Tsagou et al. 2004). This new approach meets the growing concern for new methods of pest control that do not pollute and are more environmentally friendly (Lacey et al. 2001; Meikle et al. 2012).

Different species of entomopathogenic bacteria have been isolated and used successfully for the regulation of many arthropod pests. The most investigated species are spore-forming bacteria, particularly those found in the Bacillaceae family, such as *Bacillus thuringiensis* (Bt), *B. sphaericus* and *Paenibacillus popilliae*, because they can more easily infect healthy hosts (Aronson et al. 1986; de Maagd et al. 2003). Of these species, *B. thuringiensis* is the most widely used as an agent for the biological control of pests (Schnepf et al. 1998; Lacey et al. 2001).

In sporulation, *B. thuringiensis* produces parasporal crystals consisting of proteins called δ-endotoxins, which are classified into two families: Cry and Cyt, which have insecticidal activity against different insect orders, such as Lepidoptera, Diptera, Coleoptera, Hymenoptera, Homoptera, Orthoptera and Mallophaga, as well as other organisms, such as nematodes and mites (Schnepf et al. 1998). δ-endotoxins are specific, with no toxicity to humans and other organisms (Bechtel and Bulla 1976; Bravo et al. 2013; de Maagd et al. 2003; Griffitts and Aroian 2005).

B. thuringiensis has been isolated from soil, plant leaves, the dust of stored products, and dead insects, among other habitats (Aronson 1993; Höfte and Whiteley 1989; Schnepf et al. 1998). A large number of *B. thuringiensis* isolates are selected and identified annually to find new strains with high levels of activity against arthropod pests.

The objective of this study was to isolate strains of *B. thuringiensis* from dead adult females of *V. destructor*, evaluate their pathogenicity and virulence against mites, and determine their pathogenicity toward adults and larvae of *A. mellifera* worker bees.

2. MATERIALS AND METHODS

2.1. Isolation of bacteria from dead adult females of *Varroa destructor*

Dead adult females of *V. destructor* were collected from the bottom board and some from capped brood of hives not treated with miticides; with the help of a camel hair brush they were taken and placed individually in sterile 1.5 mL microcentrifuge tubes (Tsagou et al. 2004). They were then disinfected with 2 % sodium hypochlorite for 10 s and washed twice with sterile water, and placed in microcentrifuge tubes (one mite per tube). Five hundred microliters of liquid Luria-Bertani (LB) medium was added and the contents were macerated with a sterile pipette tip, and then incubated for 72 h at 30 °C. Finally, a loop of sporulated culture was striated into Petri dishes containing solid LB to obtain single colonies.

2.2. Selection, preservation and multiplication of bacterial isolates

Using a compound microscope, we selected rod-shaped bacteria with a spore and a parasporal crystal. These characteristics correspond to *B. thuringiensis* (Bechtel and Bulla 1976).The isolates were stored in

liquid LB medium plus 60 % glycerol at −20 °C in the collection of the Laboratory of Plant Parasitology at the Center for Biological Research of the Autonomous University of the State of Morelos, Mexico. The bacterial isolates were grown on solid LB medium for 72 h at 30 °C. The culture was recovered in 1 mL of sterile distilled water and 1 mM PMSF (phenyl methanesulfonyl fluoride) was added; total protein was quantified by the Bradford technique (Bradford 1976).

2.3. Pathogenicity bioassays against *Varroa destructor*

V. destructor young adult females were collected from drone and worker capped broods of five colonies of European honey bees. The combs were taken to the laboratory, and the drone and worker brood cells were opened. Female mites were collected with a camel hair brush and placed in sterile Petri dishes with a diameter of 9 cm. Each Petri dish contained one white-eyed pupa for holding five test mites, and these mites were used immediately for the bioassays (Tsagou et al. 2004).

Twenty-two isolates of *Bacillus*-like bacteria were evaluated (EA1, EA3, EA6, EA8, EA9.3, EA11.3, EA11.2.1, EA18.1.2, EA22.1, EA23, EA26.1, EA26.2, EA27.1, EA28, EA32.2.1, EA36.2, EA40.2, EA43, EA49.1, EA49.2.1, EA88.3, EA93). These isolates were selected according to their protein expression displayed on 10 % polyacrylamide gels. In all evaluations, 100 μg/mL of total protein was used. Water and surfactant (25 % alkyl phenol polyoxyethylene ether, 15 % antifoam agent, 74 % diluent) were used as a control.

Groups of 30 *V. destructor* individuals were immersed in the indicated concentration (100 μg/mL) for 5 s, and then were cast with sterile plastic mesh. Excess of moisture was removed from the mites with filter paper (Whatman No. 1). Subsequently, in microcentrifuge tubes (1.5 mL) five mites were placed on fresh white-eyed honeybee pupae, sealed with cotton and incubated at a temperature of 34 °C (Rodriguez et al. 2009). Six replicates, each with five young adult mites, were used for isolate and mortality was monitored every day for 7 days. The mites were considered dead when they no longer showed movement when stimulated with a brush (Shaw et al. 2002). The experimental design was completely randomized.

2.4. Species identification of EA3, EA11.3 and EA26.1 *Bacillus* isolates

Total DNA was purified from each isolate using the Gentra® Puregene® Yeast/Bact. Kit, according to the manufacturer's instructions (QIAGEN, Germany). The 16S rRNA gene fragment was amplified with the primers reported by Aguino del Muro and Priest (1993) (direct BSH1 GCG CAA GCT TAG AGT TTG ATC CTG GCT CAG GAC G and reverse BSB2 GCG GAT CCT ACC TTG TTA CGA CTT CAC CCC AG) as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation (95 °C, 35 s), annealing (62 °C, 42 s), and extension (72 °C, 1:30 min) and a final extension at 72 °C for 15 min. The PCR products were separated by electrophoresis on a 1 % (w/v) agarose gel, and bands were excised and purified using a QIAquick® Gel Extraction kit (QIAGEN, Germany). The PCR products were sequenced at the facilities of the Biotechnology Institute of the National Autonomous University of Mexico. 16S rRNA sequences were analyzed using the blastn program of the GenBank database, National Institutes of Health.

2.5. Virulence bioassays against *Varroa destructor*

The results of the pathogenicity test, showed that EA3, EA11.3 and EA26.1 strains were those that produced higher mortality on *V. destructor*. As a result, these three strains were used to calculate the Lethal Concentration 50 (LC_{50}). Based on preliminary results, the strains were evaluated at different concentrations: the EA3 strain (0, 4, 8, 12, 16 and 20 μg/mL), EA11.3 (0, 10, 40, 70 and 100 μg/mL) and EA26.1 (0, 1, 2, 8 and 10 μg/mL). Treatments were applied as described in the pathogenicity tests (Shaw et al. 2002). For this assay, a total of 30 young adult mites for concentration were used on 15 replicates, each with two mites placed on white-eyed pupa, and mortality was monitored every 12 h for 7 days (Meikle et al. 2006; Tsagou et al. 2004).

2.6. Pathogenicity by consumption bioassays against *Apis mellifera* adult

The pathogenicity of two isolates of *Bacillus*-like strains (EA3 and EA26.1) was evaluated because their LC_{50} were the lowest with the following protein

concentrations: 1, 5, 25, 50 and 100 μg/mL. Three replicates of 30 bees were used for each concentration, with a total of 90 bees (a total of 450 adult bees were used for each strain) (Shaw et al. 2002).

Combs containing mature worker bee pupae were removed from two mite-free colonies and maintained at 34 °C. Groups of 10 newly emerged adult bees (>24 h old) were transferred to plastic containers (clear plastic tumblers of 14 oz); four square centimeters were removed and covered with mosquito mesh for ventilation (Evans et al. 2009). The experimental unit was three plastic containers with 10 bees each. Two hours later, the bees were fed with different concentrations of bacterial protein mixed in a solution of 50 % sucrose and 0.1 % surfactant. The solution was deposited into a feed device on the top of the plastic tumbler, and delivered ad libitum (Evans et al. 2009; Higes et al. 2007; Papadopoulou-Karabela et al. 1992; Ramirez-Romero et al. 2008; US EPA 1996). Two control groups were used: one containing only 50 % sucrose (1) and one with 50 % sucrose and 0.1 % surfactant (2) (25 % alkyl phenol polyoxyethylene ether, 15 % antifoam agent, 74 % diluent). The bees were kept in an incubator at 34 °C, mortality was measured on the seventh day by counting the number of dead bees, those that did not move when stimulated with a camel hair brush for 15 s (Evans et al. 2009; Higes et al. 2007; Lindberg et al. 2000; US EPA 1996).

2.7. Pathogenicity by dip bioassays against *Apis mellifera* adults

The pathogenicity of two strains of *Bacillus*-like strains (EA3 and EA26.1) was evaluated with 100 μg/mL of protein concentration plus 0.1 % surfactant. Combs containing mature worker bee pupae were removed from two mite-free colonies and maintained at 34 °C. Groups of 10 newly emerged adult bees (>24 h old) were transferred to plastic containers (clear plastic tumblers of 14 oz); four square centimeters were removed and covered with mosquito mesh for ventilation (Evans et al. 2009). The experimental unit was three plastic containers with 10 bees each. Two hours later, plastic containers with bees were stored in a refrigerator maintained at 4 °C until the bees showed the first signs of immobility (Scheiner and Arnold, 2010). Bees were immersed individually in the indicated concentration (100 μg/mL) for 5 s using dissecting forceps. A total of 90 adult bees were used; there were three replicates for each strain (the experimental unit was three plastic containers with 10 bees each) (Shaw et al. 2002). Two control groups were used: control 1, bees immersed in water; and control 2, bees immersed in water plus 0.1 % surfactant (25 % alkyl phenol polyoxyethylene ether, 15 % antifoam agent, 74 % diluent). The bees were kept in an incubator at 34 °C for 7 days and were fed with a solution of 50 % sucrose in a feed device located on the top of the plastic tumbler, and delivered ad libitum. Mortality was measured on the seventh day (Evans et al. 2009; Higes et al. 2007; Lindberg et al. 2000; US EPA 1996).

2.8. Pathogenicity bioassays against larvae of *Apis mellifera*

The larval diet was prepared according to Kaftanoglu et al. (2010) and the diet was divided into 2 mL centrifuge tubes and kept at −18 °C in a freezer until they were used. The diet was thawed and brought to 34 °C in a water bath just before feeding. Five concentrations of protein (1, 5, 25, 50 and 100 μg/mL) of the EA3 and EA26.1, strain were dissolved in the larval diet. A total of 450 larvae of 2.5–3 days old were used; there were five treatment groups (90 larvae per treatment), with three replicates, and 30 larvae in each replicate. One control group free of bacterial complex was used. The exposure to the protein was in the first and second day of feeding; for this, six aliquots of 10 μL of larval food with protein concentrations were placed in a polyethylene Petri dish (85 mm in diameter) and five larvae were grafted on each aliquot. After the third day, the larvae were fed as Kaftanoglu et al. (2010). The Petri dishes were transferred into a humidity chamber, and kept there at 34 ° C and 90 % RH. On the seventh day, when the larvae stops feeding, total mortality was quantified (Aupinel et al. 2005; Kaftanoglu et al. 2010). In order to discard the production of β-exotoxins on the two *Bacillus*-like strains (EA3 and EA26.1) bioassays with *Musca domestica* larvae were performed (Mac Innes and Bouver 2009) (see supplementary material).

2.9. Statistical analysis

The distribution of values obtained in each pathogenicity bioassay were analyzed by ANOVA

and Kruskal-Wallis tests, and multiple comparison of means was performed using Tukey's test, with a significance level of 0.05. In these cases, we used the SAS statistical program version 9.0. Angular transformation of data was performed previously. To determine the virulence of each strain against *V. destructor*, the LC_{50} were calculated using Probit analysis via the Polo Plus 2003 statistical program (Robertson et al. 2003).

3. RESULTS

3.1. Isolation of bacteria from corpses of *Varroa destructor*

From a total of 250 dead adult *V. destructor* females, 54 *Bacillus*-like strains were isolated. In all, the isolates were assigned a consecutive number prepending the initials EA.

3.2. Pathogenicity bioassays against *Varroa destructor*

Using a dose of 100 μg/mL, all of the *Bacillus*-like isolates evaluated were pathogenic to *V. destructor*. However, nine isolates were more pathogenic producing mortality rates above 80 % at 48 h after treatment (Figure. 1). The best results were obtained with isolate EA49.1, which showed a major protein at a molecular weight of 100 kDa, and yielded 100 % mite mortality. The EA11.3 isolate, with the major protein of 95 kDa, produced 93 % mortality, EA26.1 showed a protein of approximately 75 kDa, with 90 % mortality and EA3 revealed a major protein of approximately 95 kDa and recorded 86.7 % mortality of *V. destructor* mites. The analysis of variance for the pathogenicity of the different *Bacillus*-like isolates on *V. destructor* demonstrated significant differences among treatments ($P<0.0001$).

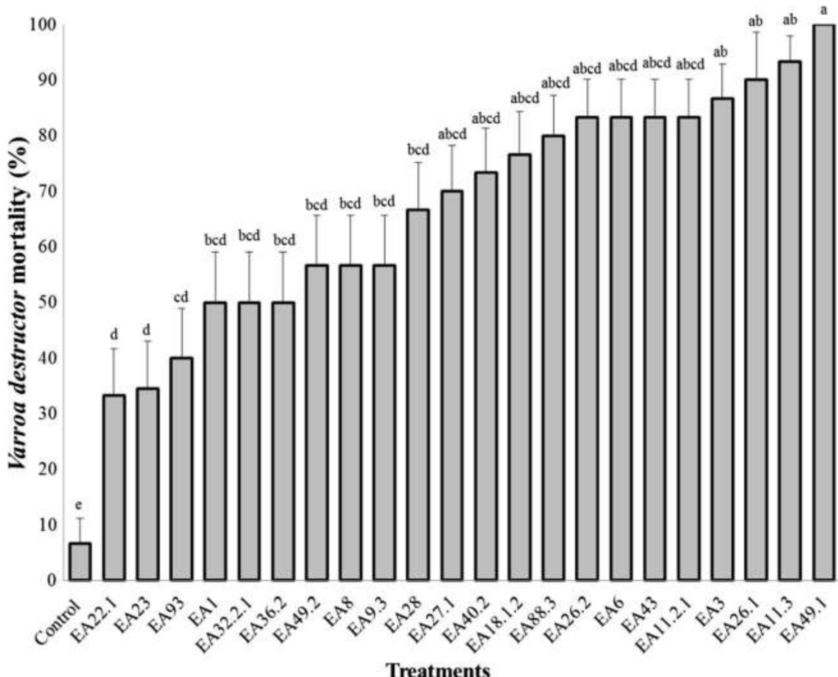

Figure 1. Mortality (±SE) of *Varroa destructor* 48 h after treatment at 100 μg/mL by different *Bacillus*-like isolates. The different letters above the bars indicate differences among treatments as determined by Tukey's test ($P<0.05$).

Table I.. Lethal Concentration 50 and 90 (μg/mL) of EA3, EA11.3 and EA26.1 isolates of *Bacillus thuringiensis* against *Varroa destructor*.

Isolate	Slope	Intersection	χ^2	LC_{50}	CI 95%[a] LC_{50}		LC_{90}	CI 95%[a] LC_{90}	
					[b]Lower L	[c]Upper L		[b]Lower L	[c]Upper L
EA3	3.557±0.740*	−3.031	1.84	7.111	5.104	8.785	16.302	12.833	25.565
EA11.3	1.770±0.389	−2.405	1.47	22.839	11.582	33.802	120.971	76.853	304.891
EA26.1	1.717±0.372	−0.307	1.28	1.509	0.721	2.324	8.413	5.217	21.309

*Standard error, [a] Confidence intervals, [b] Lower limit, [c] Upper limit

3.3. Species identification of EA3, EA11.3 and EA26.1 *Bacillus*-like isolates

16S rRNA genes were sequenced and compared with the sequences available in GenBank. BLAST searching allows for rapid searching of related sequences. The isolates, EA3 and EA11.3, showed 100 % identity with the *B. thuringiensis* serovar *tenebrionis* strain MMML-49, and EA26.1 matched 100 % with the *B. thuringiensis* serovar *kurstaki* strain HD73. The 16S rRNA gene sequences of isolates EA3, EA11.3 and EA26.1 were deposited in GenBank with the following accession numbers: KC758845, KC758846 and KC758847, respectively.

3.4. Virulence bioassays against *Varroa destructor*

Three *B. thuringiensis* isolates (EA3, EA11.3 and EA26.1) that killed more than 80 % of the mites at 100 μg/mL were selected and the LC_{50} (Lethal Concentration to kill 50 % of the population) was determined for each (Table I). EA26.1 isolate produced 96.7 % mortality at 36 h after treatment, and the LC_{50} was 1.5 μg/mL (χ^2= 1.28). The EA3 isolate produced 93.3 % mortality at 48 h after treatment, and the LC_{50} was 7.1 μg/mL (χ^2=1.84). The EA11.3 isolate produced 93.3 % mortality 60 h after treatment, and the LC_{50} was 22.8 μg/mL (χ^2=1.47). The results of this study indicate that the EA26.1 isolate was more effective because it killed more mites in relation to the EA11.3 and EA3 isolates at lower protein concentrations.

3.5. Pathogenicity bioassays against adult and larvae of *Apis mellifera*

After *A. mellifera* adults had been ingesting the protein concentrations for 7 days, the mortality rates recorded with the EA3 isolate were considerably lower (between 2.2 and 5.6 %), but higher mortality was observed in the second control (6.7 %) (Figure. 2). When the mortality among treatments was compared, we observed significant differences between the bees of the control group

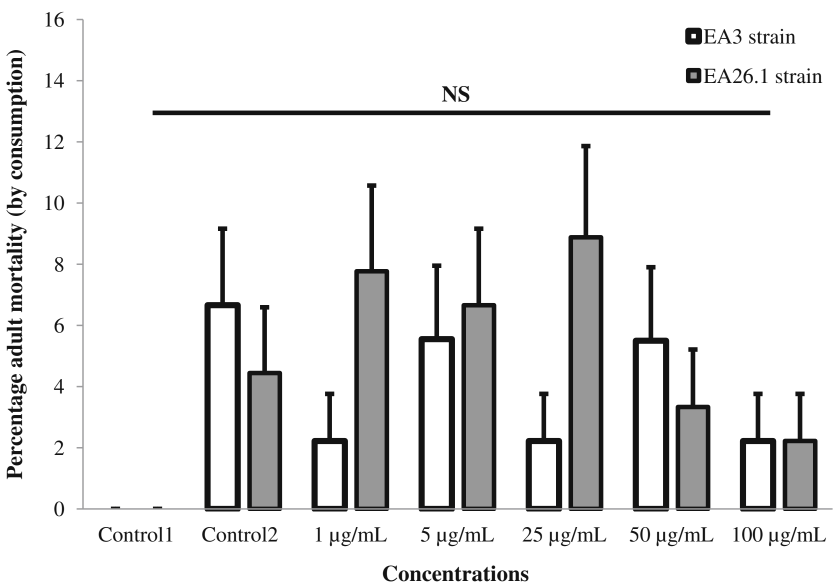

Figure 2. Percentage mortality (±SE) of adult bees 7 days after being fed different protein concentrations of the EA3 (*white bars*) and EA26.1 (*grey bars*) strains. Control 1, bees fed with 50 % sucrose, and control 2, bees fed with 50 % sucrose plus 0.1 % surfactant.

that did not receive surfactant and the control group that did receive surfactant (second control), but there were no significant differences among five treatments and controls (χ^2=13.24; 6 df; P= 0.039). The EA26.1 isolate caused mortality between 2.2 and 8.9 % (Figure. 2), but there were no significant differences among five treatments and controls (χ^2=11.05; 6 df; P=0.088).

Also, the mortality of adult bees treated by dip was recorded. The results with both strains (EA3 and EA26.1) showed less than 15 % mortality, and no significant difference was found compared with the two control groups (χ^2=6.71; 3 df; P=0.08) (Figure 3).

The larvae that were fed different concentrations of total protein of the EA3 strain, showed a percentage of mortality between 18 and 32 %. These are high mortalities, but the control larvae group also showed a mortality of 17 % (Figure. 4). No significant difference was found among the treatments and the control group (χ^2=2.29; 5 df; P=0.80). The larvae that were fed with different concentrations of total protein of the EA26.1 strain, showed a percentage of mortality between 19 and 28 % (Figure. 4). In this case, the control group showed a mortality of 18 %. No significant difference was found among the treatments and the control group (χ^2=1.59; 5 df; P=0.90).

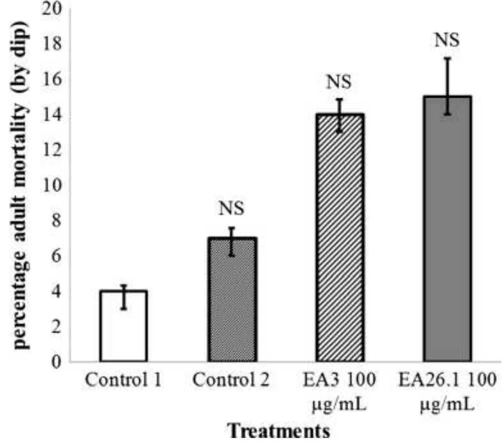

Figure 3. Percentage mortality (±SE) of adult bees 7 after dip at 100 µg/mL of the EA3 and EA26.1 strains. Control 1, bees immersed in water, and control 2, bees immersed in water plus 0.1 % surfactant. NS: not significant from the control group (Tukey's test; P<0.05).

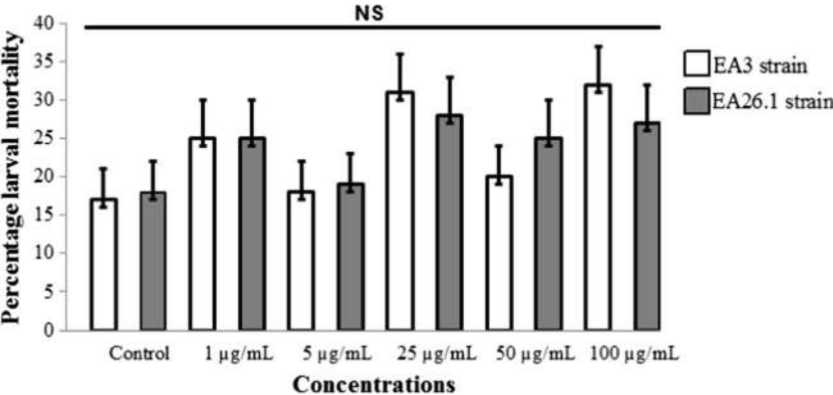

Figure 4. Percentage larval mortality (±SE) 7 days after being fed with larval diet plus different protein concentrations of the EA3 (*white bars*) and EA26.1 (*grey bars*) strains. NS: not significant from the control group (Tukey's test; $P<0.05$).

4. DISCUSSION

V. destructor is the most serious pest for *A. mellifera*, and some researchers have noted that it plays an important role in the phenomenon called Colony Collapse Disorder (De Rycke et al. 2002; Martin 2001; Topolska et al. 2008). Unfortunately, *V. destructor* has developed resistance to miticides, so there is a desperate need to develop new ways to control this parasitic mite.

The results of this study show that nine *Bacillus*-like isolates tested for pathogenicity and three tested for virulence have a lethal effect against *V. destructor* in short time periods (36, 48 and 60 h). Tsagou et al. (2004) isolated one *Bacillus* sp. strain from *V. destructor* which was evaluated against the same mite using 0.5 mL of extracellular broth and cellular extract, without quantifying protein. They report virulence with an LT_{50} (Lethal Time to kill 50 % of the population) 4 h for both and an LT_{90} of 21 and 43 h, respectively. Although, we did not calculate LT, more than 90 % of the mites were killed at 36 h (EA26.1) and 48 h (EA3), so these isolates also kill the mites quickly.

Like Tsagou et al. (2004) we did not know which factors were involved in toxicity against *V. destructor*. Peña et al. (2006) reported a protein of a molecular weight of 100 kDa toxic to *Epilachna varivestis* (Coleoptera), produced by a strain of *B. thuringiensis* which is an S-layer type. EA49.1 produces one of a similar weight. Ruiz-de Escudero et al. (2006) reported a protein Cry1Ia7 with a molecular weight of ~75 kDa, like the EA26.1 isolate, and it was toxic to *Letipnotarsa decemlineata*. However, it is possible that δ-endotoxins (Cry and Cyt) may have played a role since they are known to be responsible for the insecticidal activity of *B. thuringiensis* on a large number of different insect species (Bravo et al. 2007). After 24 h of treatment, some mites were trembling; also, regurgitation and dysentery were observed, this suggest that the activity of the proteins of these *B. thuringiensis*, could be similar to the reported for Cry1 toxins by Bravo et al. (2004).

Several reports have shown that when a *B. thuringiensis* strain is isolated from corpses, it is pathogenic to the same insect species. Angus and Norris (1968) isolated three strains of *B. thuringiensis* from corpses of *Bombyx mori* L., and all were toxic to this insect species; the strain HD1 of *B. thuringiensis* was isolated from a corpse of *Pectinophora gossypiella* (Saunders) by Dulmage (1970); it is the active ingredient of all commercial bioinsecticides, and is toxic to more than 100 species of Lepidoptera. Krieg et al. (1983) isolated from *Tenebrio molitor* L. dead larvae a *B. thuringiensis* isolate that is toxic to several species of Coleoptera.

On the other hand, biosafety tests on beneficial insects are very important and are required

before the introduction of an entomopathogenic into the field to control a pest.

The results of our studies show that the strains used in this study are not pathogenic in vitro to adults and larvae of *A. mellifera*; bees were subjected to high concentration of total protein of *B. thuringiensis*. In the case of the tests made with the EA3 strain, bees were treated with a concentration 14 times the LC_{50} found for *V. destructor*. In the case of the tests made with the EA26.1 strain, bees were treated with a concentration 67 times the LC_{50} found for *V. destructor*. In all cases, the mortality of bees was not significant compared to the control groups. *Bacillus thuringiensis* produces Cry1 proteins that are pore-forming toxins; upon ingestion, the crystals are solubilized and activate by midgut proteases, monomers bind cadherina-like receptors, the α-1 is cleaved and oligomeric pre-pore structure is formed, then the oligomers bind to APN (aminopeptidase N) and move to the DMR (detergent-resistant membrane microdomains) it inserts into the membrane and forms the pore that kills the cells (Bravo et al. 2004). However, not all insect species have the same receptors or midgut proteases; these could make them susceptible or resistant to *B. thuringiensis* toxins (Gomez et al. 2014). Honey bees are fed mainly carbohydrates and proteins, and also lipids, vitamins and minerals (Brodschneider and Crailsheim 2010), so they could have different midgut proteases which inactivate the *B. thuringiensis* proteins that are toxic to the adults of *V. destructor*.

Something that is important to note in this study is that, in all tests on adults and larvae, the mortality period of observation was 7 days, which is longer than that specified by the Office of Prevention Pesticides and Toxic Substances (OPPTS) and the United States Environmental Protection Agency (EPA), which indicates that the test duration is 48 h (US EPA 1996). This suggests that bees (including larvae) are tolerant to high concentrations of EA3 and EA26.1 strains of *B. thuringiensis*.

The EA3 and EA26.1 isolates produced less than 15 % mortality in adult bees, but when we compare the mortality from the control groups, we see that the surfactant-free controls (control 1) showed mortalities of 0 % in the diet treatments and 4 % the dip treatments. Contrary to this, the control groups containing surfactant (control 2) showed 7 % mortality in both tests (diet and dip treatments). This suggests that some components of the surfactant could be toxic to bees (Timothy et al. 2012). Therefore, special testing is needed to see if the surfactants used in this study produced adverse effects on honey bees.

There is concern about using *B. thuringiensis* strains producing β-exotoxins as a bioinsecticide, because it is known that these toxins are highly toxic to insects, and in some cases to mammals, and also are mutagenic because of its structural analogy to ATP (Šebesta et al. 1981). The mortality caused to adult *V. destructor* by EA3 and EA26.1 *B. thuringiensis* strains are not due to β-exotoxins which are thermostable, because no mortality or pupae malformation was observed on the larvae of *M. domestica* treated with the autoclaved supernatants (see supplementary material). So these strains could be used to control *V. destructor* without any adverse effects on bees.

To date, research regarding the effect of *B. thuringiensis* in bees has focused on toxins that are expressed in genetically engineered plants. These studies indicate that the Cry1Ab, Cry1Ac, Cry1Ah and Cry1Ba toxins of *B. thuringiensis* are not lethal toward adult bees. Despite several days of feeding a diet contaminated with toxins, the survival rates are upwards of 80 % (Babendreier et al. 2005; Malone et al. 1999; Han et al. 2010; Ping-Li et al. 2012; Ramirez-Romero et al. 2008). So far, there are no reports indicating that the Cry proteins of *B. thuringiensis* are directly toxic to *A. mellifera* (Chilcutt and Tabashnik 1999; Duan et al. 2008; Walker et al. 2007).

Additionally, bees maintain a wide range of bacterial taxa in their digestive system, which are capable of inhibiting the growth of pathogens such as *Paenibacillus larvae* (Evans and Tamieka-Nicole 2006). The majority of bacteria in the digestive tract belong to the genus *Bacillus*, suggesting a stable symbiosis between bees and this bacterial taxon. This could explain why bees are more tolerant to *B. thuringiensis* than other insects (Evans and Tamieka-Nicole 2006; Gilliam 1997, 1978).

Despite the huge problem that *V. destructor* represents in beekeeping, research studies on its biological control are very limited. Therefore, it is necessary to continue these investigations not only in laboratory conditions, but also in field conditions.

ACKNOWLEDGEMENTS

We thank Marisa Mendoza Flores for providing the hives infested with *V. destructor*. E. V.A.R. acknowledges the Consejo Nacional de Ciencia y Tecnología for the PhD fellowship, and the four anonymous reviewers for the improvement of the manuscript.

Susceptibilité *in vitro* de *Varroa destructor* et d'*Apis mellifera* à des souches naturelles de *Bacillus thuringiensis*

Apidae / pathogénicité / virulence / Acari / ectoparasite

In vitro-Empfindlichkeit von *Varroa destructor* und *Apis mellifera* gegenüber einheimischen Stämmen von *Bacillus thuringiensis*

***Varroa destructor / Apis mellifera / Bacillus thuringiensis* / Pathogenität / Virulenz**

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