Original Article

# EFFECTS OF BACTERIAL CELL-FREE SUPERNATANT ON NUTRITIONAL PARAMETERS OF *APIS MELLIFERA* AND THEIR TOXICITY AGAINST *VARROA DESTRUCTOR*

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**Abstract**

***Apis mellifera* L. is an essential pollinator that is currently being affected by several stressors that disturb their ecological function and produce colony losses. Colonies are being seriously affected by the ectoparasitic mite *Varroa destructor*. The relationship between stressors and bee symbionts is being studied in order to enhance bee health. The goal of this study was to evaluate the effect of cell-free supernatants (CFSs) produced by *Lactobacillus johnsonii* AJ5, *Enterococcus faecium* SM21 and *Bacillus subtilis* subsp. *subtilis* Mori 2 on *A. mellifera* nutritional parameters and their toxicity against *V. destructor*. Toxicity and survival bioassays were conducted on adult bees with different concentrations of CFSs. Nutritional parameters such as soluble proteins and fat bodies in abdomens were measured. *Varroa destructor* toxicity was analyzed by a contact exposure method and via bee hemolymph. At low concentrations, two of CFSs tends to enhance bee survival. Remarkably fat bodies maintained their levels with all CFS concentrations in the abdomens, and soluble protein increased at a high concentration of two CFSs. Toxicity against *V. destructor* was observed only via hemolymph, and results were in agreement with the treatment that produced an increase in bee proteins. Finally, CFS produced by *L. johnsonii* AJ5 could be a promising natural alternative for strengthening bee health.***

**Keywords: *Apis mellifera*, bacterial metabolites, beneficial bacteria, nutrition, *Varroa destructor***

## INTRODUCTION

In their role as pollinators, honey bees are essential for agriculture, and therefore for the worldwide economy (Watson & Stallins, 2016), however colony loss syndrome, always on the increase, remains a serious issue (Lee et al., 2015; Seitz et al., 2015). The scientific community agrees that this scenario could be explained through a combination of such stressors (e. g. Neumann & Carreck, 2010) as pathogens (Bahreini & Currie, 2015), pesticides use (Kakumanu et al., 2016), monocultures (Maggi et al., 2016), climate change and invasive species (Nieto et al., 2014).

The ectoparasitic mite *Varroa destructor* has become the main pest of *Apis mellifera.* This mite feeds on the hemolymph of bees (e. g. De D'Aubeterre et al., 1999), although in recent studies Ramsey et al. (2018, 2019) has proposed that it feeds on bee fat body. However, the damages remain the same, a consequent reduction of protein content (Tewarson, 1983) and hemocytes (Amdam et al., 2004) leading to an imbalanced gut microbiota (Hubert et al., 2017) and decreased host's immune response (Gregory et al., 2005). In addition, *Varroa* mites act as a virus-vector (Antúnez et al., 2015). Scientists (e. g. Simion et al., 2011; Medici et al., 2015)have been alarmed by increasingly restricted parasite control and increasing acaricide resistance and residues in beehive products have resulting in several EU countries banning some of these compounds (European Commission, 2010) and seeking natural alternatives. The focus is on the mutualistic relationship between bees and their microbiota (e.g. Crotti et al., 2013; Alberoni et al., 2016). Commensal bacteria contribute to an increase in nutrient availability (Crotti et al., 2012) through the metabolism of toxic carbohydrates (Newton et al., 2013), the degradation of food components (Kwong & Moran, 2016; Kesinerova et al., 2017) as well as pesticides and antibiotics. Moreover, these supply part of the fatty acid, amino acid, metabolite and vitamin bee demands through microbiota secondary products (Brodschneider & Crailsheim, 2010; Crotti et al., 2013). Gut symbionts are involved in the stimulation of the host's immune system (Caccia et al., 2016) and contribute to the first line of bees' defense of biofilm formation (Engel et al., 2012) by competing with microorganisms or by secreting antimicrobial compounds (Crotti et al., 2013; Alberoni et al., 2016). There is strong evidence that they induce an increase of antimicrobial peptide (AMPs) (Jefferson et al., 2013; Janashia & Alaux, 2016). *In vitro* trials have confirmed the ability of lactobacilli and bifidobacteria to inhibit honey bee pathogens (Audisio et al., 2011; Vásquez et al., 2012). Similarly, a reduction in *N. ceranae* intensity has been reported with the supply of *Bacillus* spp. (Sabaté et al., 2012) or their metabolic products (Porrini et al., 2010), a mix of bifidobacteria and lactobacilli (Baffoni et al., 2016) and *Lactobacillus* spp. (Maggi et al., 2013). Other evidence also supports a lower incidence of *V. destructor* in colonies supplied with lactobacilli (Audisio et al., 2015) or *Bacillus subtilis* subsp. *subtilis* Mori2 (Sabaté et al., 2012).

The balance of the beneficial microbiota can be disturbed by the combination of different stressors (Audisio, 2016; Kakumanu et al., 2016). Researchers have been joining efforts to determine the beneficial effects of symbionts and how to utilize them to improve bee health and thus colony performance (e.g. Alberoni et al., 2016; Audisio, 2016). In this scenario, bacterial metabolites appear as an ecological and environmentally-friendly alternative (Crotti et al., 2012; Moran, 2015). In the present study, *L. johnsonii* AJ5, *E. faecium* SM21 and *B. subtilis* subsp. *subtilis* Mori2 strains were selected based on their effects reported previously (Audisio & Benítez-Ahrendts, 2011; Sabaté et al., 2012). The purpose of this study was to determine the effect of bacterial cell-free supernatant on *A. mellifera* L., bee nutritional parameters, and their toxicity against *V. destructor* mite.

## MATERIAL AND METHODS

### Biological material

Experiments were conducted in EEA-INTA Balcarce, Argentina (37°45'42.5"S 58°18'04.3"W). Trials were carried out with

newly emerged (24-48 h) *A. mellifera* L. bees obtained from sealed brood combs placed in an incubator at 33 ± 1.5 °C and 70 ± 3 % of relative humidity (RH), collected from colonies free of the main pathologies.

**Bacterial strain and metabolite synthesis**

*L. johnsonii* AJ5 and *E. faecium* SM21 were isolated from *A. mellifera* L bee gut and grown on MRS and BHI broth (Britania, Argentina) respectively, and *B. subtilis* subsp. *subtilis* Mori 2 was isolated from honey in Salta (INIQUI, UNSa, Salta 24°43'39"S 65°24'28"W) and grown on BHI broth. Bacterial metabolites synthesized by the strains were recovered as cell-free supernatants (CFS) after each culture was centrifuged and filter-sterilized, as described by Audisio & Benítez-Ahrendt (2011) and Sabaté et al. (2009).

**Metabolite characterization and quantification**

The organic acids produced by *L. johnsonii* AJ5 were characterized and quantified by HPLC, bacteriocins synthesized by *E. faecium* SM21 were determined using *Listeria monocytogenes* 01/155 as the indicator culture (Audisio (Audisio & Benítez-Ahrendts, 2011et al., 2011) and lipopeptides produced by *B. subtilis* subsp. *subtilis* Mori 2 were determined by ultraviolet matrix-assisted laser desorption-ionization mass spectrometry (UV-MALDI MS) performed on a Bruker Ultraflex Daltonics Time-of-Flight/Time-of-Flight (TOF/TOF) mass spectrometer (Leipzig, Germany) (Torres et al., 2015).

**Survival of honey bees against bacterial metabolites**

Honey bees were individually confined in plastic containers (3 x 3 cm) and supplied on a daily basis with 80 µl of each CFS concentration in syrup 2:1 (water:sugar). Two control groups were performed: sugar syrup and a solution of culture media and syrup (6.25 % v/v MRS and 15 % v/v BHI). The concentration of different CFSs in syrup was 1, 6, 20 and 40% v/v for CFS1 (*L. johnsonii* AJ5); 1, 5, 15 and 30 %v/v for CFS2 (*E. faecium* SM21) and 5, 15, 30 and 60 %v/v for CFS3 (*B. subtilis* subsp. *subtilis* Mori 2). Each treatment was replicated thirty times. Daily bee mortality was also registered for six days and survival curves were built.

**body and soluble protein determination**

Groups of twenty-five newly emerged bees (24 - 48 h, for 3 blocks) were kept in cages and fed ad libitum with the above-mentioned concentrations of CFS for six days, and after that twenty bees were killed in liquid nitrogen and then kept at -80°C up to be analyzed.

Soluble protein determination. Abdomens were individually ground-glass homogenized in 1 mL of distilled water and then centrifuged at 1500 g for 5 min. Aliquots of supernatant were tested for soluble protein by the dye-binding method of Bradford (1976) using BioRad Dye Reagent (BioRad Labs. GmbH) and bovine serum albumin (Sigma [fractionV, 98%]) as standard (Bowen-Walker & Gunn 2001).

Fat bodies determination. Adult abdomens were dried out for five days at 36 °C and then they were weighed and washed in ethyl ether for 24 h for fat to be dissolved. The abdomens were then dried out for three days and weighed again. Fat bodies were calculated as the change in abdominal weight after the ethyl ether wash (Wilson-Rich et al., 2008).

**Bioassays to determine the effects of CFS on *V. destructor***

Contact exposure method. *V. destructor* female adults were obtained from brood cells, and six (for 5 replicates) were placed for 1 min on a piece of filter paper (3 x 3 cm) previously impregnated with 200 µL of 100 %, 60 %, 30 % and 10 % v/v CFS/distilled water and then were transferred to glass Petri dishes (modified from Damiani et al., 2010). Control groups consisted in culture media and distilled water. Mortality was counted every hour up to seven hours.

through the bee. Three **newly emerged bees placed in** glass Petri dishes were fed *ad libitum* with the CFSs concentration already mentioned in section 2.4. After three days, six female mites were incorporated per dish (for five replicates), and their mortality was registered at 24, 48, and

72 h (modified from Ruffinengo et al., 2005). Control groups consisted in bees only fed with sugar syrup and culture media.

### Data analysis

Kaplan-Meier survival curves were obtained to estimate CFSs' effect on bee survival. The differences between curves with its respective control groups were compared by using the log-rank test applying Bonferroni correction. Proteins and fat bodies were analyzed by ANOVA for a Randomized Complete Block Design (RCBD). The blocking variable was the time in which each trial was performed. Another ANOVA test was performed for each CFS effect on *V. destructor*. Mortality means between concentrations and control groups were compared with the Tukey test ($p < 0.05$). Statistical analyses were performed using R software (version 3.1.1, 2014).

## RESULTS

### Characterization and quantification of the main bacterial metabolites

The chemical nature of the main bacterial metabolites and their concentration in each CFS were estimated by HPLC and/or the titer against *L. monocytogenes* 01/155 (Audisio et al., 2005; Torres et al., 2015); CFS 1 (*L. johnsonii* AJ5): lactic acid 275 ± 8 mM, CFS 2 (*E. faecium* SM21): lactic acid 34 ± 5 mM and bacteriocins 1.066,6 UA/mL, and CFS 3 (*B. subtilis* subsp. *subtilis* Mori2): surfactins 2.000 UA/mL.

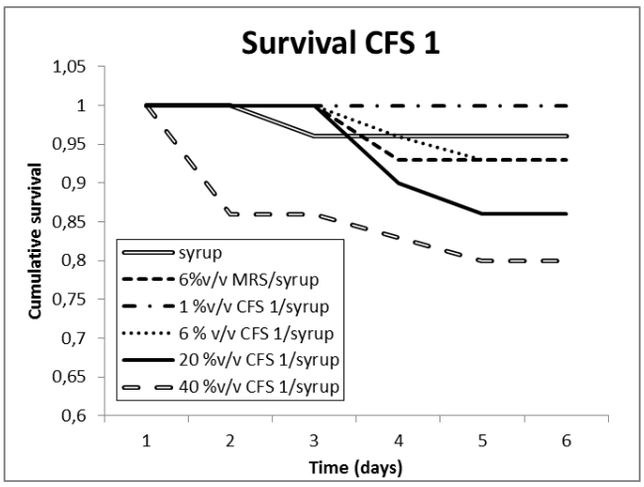


Fig. 1. Cumulative survival of bees fed on different concentrations of CFS 1 (cell-free supernatant produced by *Lactobacillus johnsonii* AJ5) for six days.

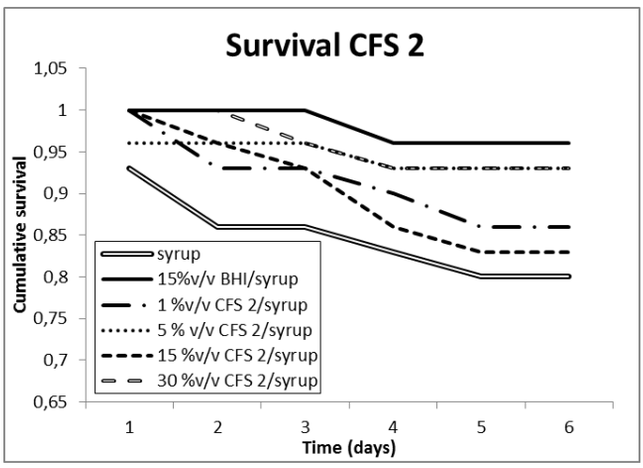


Fig. 2. Cumulative survival of bees fed on different concentrations of CFS 2 (cell-free supernatant produced by *Enterococcus faecium* SM21) for six days.

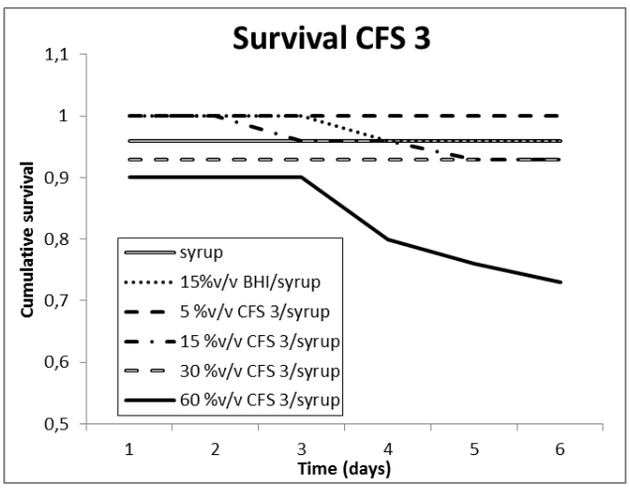


Fig. 3. Cumulative survival of bees fed on different concentrations of CFS 3 (cell-free supernatant produced by *Bacillus subtilis* subsp. *subtilis* Mori2) for six days.

### Toxicity of CFSs against honey bees

CFS 1 showed a substantial difference between CFS 1/syrup (40%v/v) against syrup control and CFS 1/syrup (1%v/v) (Kaplan-Meier Survival Analysis, $p<0.05$, Fig.1). CFS 2 shows only one significant difference between syrup control and BHI/syrup (15% v/v) (Kaplan-Meier Survival Analysis, $p<0.05$). The greatest survival to CFS 2 was detected at CFS 2/syrup (5% v/v) and CFS 2/syrup (30% v/v) (Fig. 2). CFS 3 yielded significant

Table 1.

Mean and standard deviation of soluble protein per bee (mg/mg) for each CFS concentration

| | CFS concentration | Mean ± SD |
|---|---|---|
| Control | Syrup | 0.0105$^{bc}$ ± 0.0022 |
| | MRS/syrup | 0.0098$^{c}$ ± 0.0029 |
| | BHI/syrup | 0.0106$^{bc}$ ± 0.0018 |
| CFS1 | 1 % v/v CFS 1/syrup | 0.0106$^{bc}$ ± 0.0023 |
| | 6 % v/v CFS 1/syrup | 0.0112$^{abc}$ ± 0.0027 |
| | 20 % v/v CFS 1/syrup | 0.0119$^{abc}$ ± 0.0033 |
| | 40 % v/v CFS 1/syrup | **0.0146$^{a}$ ± 0.0054** |
| CFS2 | 1 % v/v CFS 2/syrup | 0.0115$^{abc}$ ± 0.0029 |
| | 5 % v/v CFS 2/syrup | 0.0115$^{abc}$ ± 0.0036 |
| | 15 % v/v CFS 2/syrup | 0.0125$^{abc}$ ± 0.0025 |
| | 30 % v/v CFS 2/syrup | 0.0136$^{ab}$ ± 0.0049 |
| CFS3 | 5 % v/v CFS 3/syrup | 0.0110$^{abc}$ ± 0.0026 |
| | 15 % v/v CFS 3/syrup | 0.0127$^{abc}$ ± 0.0048 |
| | 30 % v/v CFS 3/syrup | **0.0142$^{a}$ ± 0.0039** |
| | 60 % v/v CFS 3/syrup | - |

Different letters represent significant differences between treatments ($p<0.05$). (-) stands for absence of sample (bees died before the end of the trial). SD= standard deviation; MRS= MRS broth; BHI= BHI broth; CFS = cell-free supernatant produced by: 1- *L. johnsonii* AJ5; 2- *E. faecium* SM21; 3- *B. subtilis* subsp. *subtilis* Mori2. Bolt letters represent the highest values.

Table 2.

Mean and standard deviation of fat body mass per bee (mg/bee) for each CFS concentration

| | CFS concentration | Mean ± SD |
|---|---|---|
| Control | Syrup | 0.0021$^{ab}$ ± 0.0012 |
| | MRS/syrup | 0.0018$^{ab}$ ± 0.0012 |
| | BHI/syrup | 0.0018$^{ab}$ ± 0.0009 |
| CFS1 | 1 % v/v CFS 1/syrup | 0.0019$^{ab}$ ± 0.0010 |
| | 6 % v/v CFS 1/syrup | 0.0018$^{ab}$ ± 0.0010 |
| | 20 % v/v CFS 1/syrup | 0.0019$^{ab}$ ± 0.0009 |
| | 40 % v/v CFS 1/syrup | 0.0020$^{ab}$ ± 0.0010 |
| CFS2 | 1 % v/v CFS 2/syrup | 0.0019$^{ab}$ ± 0.0013 |
| | 5 % v/v CFS 2/syrup | 0.0021$^{ab}$ ± 0.0017 |
| | 15 % v/v CFS 2/syrup | 0.0015$^{b}$ ± 0.0008 |
| | 30 % v/v CFS 2/syrup | **0.0022$^{a}$ ± 0.0013** |
| CFS3 | 5 % v/v CFS 3/syrup | 0.0018$^{ab}$ ± 0.0010 |
| | 15 % v/v CFS 3/syrup | **0.0023$^{a}$** ± 0.0011 |
| | 30 % v/v CFS 3/syrup | 0.0019 $^{ab}$ ± 0.0008 |
| | 60 % v/v CFS 3/syrup | - |

Different letters represent significant differences between treatments ($p<0.05$). (-) stands for absence of sample (bees died before the end of the trial). SD= standard deviation; MRS= MRS broth; BHI= BHI broth; CFS = cell-free supernatant produced by: 1- *L. johnsonii* AJ5; 2- *E. faecium* SM21; 3- *B. subtilis* subsp. *subtilis* Mori2. Bolt letters represent the highest values.

Table 3.
*V. destructor* mortality (%) at different CFS concentrations (% v/v) at 7 h of contact exposition

| | CFS concentration | Mortality % |
|---|---|---|
| Control | Water | 0 |
| | MRS/water | 0 |
| | BHI/water | 0 |
| CFS1 | 10 % v/v CFS1/water | 3.33 |
| | 30 % v/v CFS1/water | 11.1 |
| | 60 % v/v CFS1/water | 3.57 |
| | 100 % v/v CFS1/water | 6.66 |
| CFS2 | 10 % v/v CFS2/water | 0 |
| | 30 % v/v CFS2/water | 0 |
| | 60 % v/v CFS2/water | 0 |
| | 100 % v/v CFS2/water | 3.33 |
| CFS3 | 10 % v/v CFS3/water | 0 |
| | 30 % v/v CFS3/water | 0 |
| | 60 % v/v CFS3/water | 0 |
| | 100 % v/v CFS3/water | 0 |

MRS= MRS broth; BHI= BHI broth; CFS = cell-free supernatant produced by: 1- *L. johnsonii* AJ5; 2- *E. faecium* SM21; 3- *B. subtilis* subsp. *subtilis* Mori2.

differences between CFS 3/syrup (60% v/v) and syrup control, BHI/syrup (15% v/v) and CFS 3/ syrup (5% v/v), where the latter was the concentration that involved the highest pattern of bees' survival (Kaplan-Meier Survival Analysis, $p<0.05$) (Fig. 3).

**Protein and fat body determination**

*Soluble protein contents.* Results showed significant differences between concentrations of different CFSs against their control culture values (ANOVA, p=2.304e-05). Control groups displayed the lowest average of soluble protein per abdomen with values ranging from 0.0098 ± 0.0029 mg/mg to 0.0106 ± 0.0018 mg/mg. The highest soluble protein amount was detected in CFS1/syrup (40%v/v) and CFS3/syrup (30%v/v), being 0.0146 ± 0.0054 mg/mg and 0.0142 ± 0.0039 mg/mg, respectively, which were significantly different from their respective control group (Tab. 1). The other treatments did not differ respect to their control group.

*Fat bodies.* The treatment (ANOVA, p=0.04) was observed to significantly affect fat body mass, even though it was not significant between control groups and any CFS concentration tested. The highest fat body mass value was obtained at CFS2/syrup (30%v/v) and CFS3/ syrup (15%v/v), being 0.0022 ± 0.0013 mg/bee and 0.0023 ± 0.0011 mg/bee, respectively (Tab. 2).

**Effects of CFS on *Varroa destructor***

*Contact exposure method*. Mite mortality did not exceeded 10% after 7 h of exposure in both control groups and at different CFS concentrations. Only the treatment CFS 1 (30% v/v) shows mortality values over 10% (Tab. 3).

*Through the bee*. Mite mortality assays showed a lack of differences between CFSs concentrations in respect to their control group. This was observed for CFS 2 and CFS 3 with an effectiveness range of 13 to 25.9%. However, significant differences were detected at the highest concentrations of CFS 1 (40% v/v) with regards to control groups (ANOVA, p=0.00439), obtaining value of 56.6% being thus the greatest efficiency obtained in all the assays (Tab. 4).

Table 4.

Mean and standard deviation of dead mite at 72 h in contact with bees feeding with different CFSs concentrations

| | CFS concentration | Dead mite ± SD | Effectiveness (%) |
|---|---|---|---|
| Control | syrup | 0.125$^{a}$ ± 0.337 | 12.5 |
| | MRS/syrup | 0.130$^{a}$ ± 0.344 | 13 |
| | BHI/syrup | 0.133$^{a}$ ± 0.345 | 13.3 |
| CFS1 | 1 % v/v CFS 1/syrup | 0.218$^{a}$ ± 0.420 | 21.8 |
| | 6 % v/v CFS 1/syrup | 0.419$^{ab}$ ± 0.501 | 41.9 |
| | 20 % v/v CFS 1/syrup | 0.333$^{ab}$ ± 0.479 | 33.3 |
| | 40 % v/v CFS 1/syrup | 0.566$^{b}$ ± 0.504 | 56.6 |
| CFS2 | 1 % v/v CFS 2/syrup | 0.130$^{a}$ ± 0.344 | 13 |
| | 5 % v/v CFS 2/syrup | 0.214$^{a}$ ± 0.417 | 21.4 |
| | 15 % v/v CFS 2/syrup | 0.200$^{a}$ ± 0.406 | 20 |
| | 30 % v/v CFS 2/syrup | 0.166$^{a}$ ± 0.379 | 16.6 |
| CFS3 | 5 % v/v CFS 3/syrup | 0.259$^{a}$ ± 0.446 | 25.9 |
| | 15 % v/v CFS 3/syrup | 0.233$^{a}$ ± 0.430 | 23.3 |
| | 30 % v/v CFS 3/syrup | 0.241$^{a}$ ± 0.435 | 24.1 |
| | 60 % v/v CFS 3/syrup | 0.240$^{a}$ ± 0.435 | 24 |

Different letters represent significant differences between treatments ($p<0.05$). MRS= MRS broth; BHI= BHI broth; CFS = cell-free supernatant produced by: 1- *L. johnsonii* AJ5; 2- *E. faecium* SM21; 3- *B. subtilis* subsp. *subtilis* Mori2. Bolt letters represent the highest values.

## DISCUSSION

Bacterial gut symbionts and their secondary metabolites are increasingly being considered as a solution for gut microbial imbalance in bees due to their central role strengthening bees' immune system (Crotti et al., 2012; Alberoni et al., 2016). While several studies have addressed the beneficial effects of using this bacterial strain on bee health (e. g. Porrini et al*.,* 2010; Audisio et al., 2015), this study aimed to explore an alternative treatment determining the effect of their cell free supernatants.

Preliminary results of CFSs toxicity on bees suggest that no CFS is lethal after 72 h of consumption by bees. So, bees survival was higher than 0.75 in most CFS concentrations. Except for CFS 2 where all concentrations showed the lowest survival values, even the control groups CFS 1 and CFS 3 yielded the highest survival in the lowermost concentrations. These results suggest that the different CFSs at low concentrations tend to enhance or maintain bee survival as compared to control groups. On the other hand, Ptaszyńska et al. (2016) after administering a prebiotic (inulin) product did not observe any difference in bee survival. However, even though bacteria strain administration was considered as a probiotic, the authors reported low survival of bees after feeding on *Lactobacillus rhamnosus* and a mix of this probiotic with inulin. Nonetheless, several studies on colony health and beehive performance parameters revealed positive effects with probiotics as *L. johnsonii* CRL 1647 (Audisio & Benítez-Ahrendts, 2011), *B. subtilis* subsp. *subtilis* Mori2 (Sabaté et al., 2012), and prebiotics *L. johnsonii* CRL 1647 metabolite (Maggi et al., 2013). Soluble proteins and fat bodies were analyzed in order to complement toxicity and survival information in an attempt to find CFSs concentration which significantly affected bee survival. Both parameters are associated with the nutritional status of individual bees (Ament et al*.,* 2011; Nilsen et al., 2010). High nutrition standard is evidenced

by the storage of nutrients in trophocytes and oenocytes that constitute the fat body (Nilsen et al., 2010), a major storage site of lipids and proteins (de Oliveira & da Cruz-Landim, 2003) and where such proteins as vitellogenin (Corona et al., 2007; Ament et al., 2011) and antimicrobial peptides (Wilson-Rich et al., 2008) are synthesized. Fifty percent of its dry weight are lipids, which is an indicator of bee health (Arrese & Soulages, 2010). In our study, no CFSs concentration led to an increase in lipid mass on bees' abdomen, which suggests that the different CFS concentrations would maintain even bee nutritional status. These results vary with respect to those published by Maggi et al. (2013), who reported an increased fat body mass in bees of colonies supplemented with bacterial metabolites.

Two high values of abdominal soluble protein content of were obtained in CFS 1/syrup (40% v/v) and 30% v/v CFS 3/syrup treatments. This is the result of a lactic-acid rich supernatant, which along with short-chain fatty acids and acetic acids was described by Engel et al. (2012) as a bee symbiotic taxa product that could act as a supplement to the honeybee diet. Crotti et al. (2013) proposed that such bee microbiota products as acetic acid or amino acid play a role in regulating bees developmental rate, enhancing body size and improving energy metabolism. The parasitic relationship between *Varroa* and honey bees has been studied for years, but current studies are now centering on the relationship between mite and honey bee microbiota. Increasing evidence indicates that the degree of *Varroa* infestation and its chemical control affect the beneficial bacteria composition (Sandionigi et al., 2015; Hubert et al., 2017). In accordance with this matter, we tried to measure the effects of different CFSs produced by bacterias of the bees microbiota against the mite. Mite mortality at contact exposure below ten percent suggests that CFSs at those concentrations were not toxic for bees. All concentrations of CFSs administered to bees performed around 20% of efficiency and showed no significant differences with respect to control groups. However, CFS 1/syrup (40% v/v) showed 56 % of efficiency compared to control groups, which is in agreement with the high protein values obtained in bees. In some studies, where beneficial bacteria were administered to bees inside the hives, a reduction of *Varroa* incidence was detected. Márquez Gutiérrez et al. (2003) reported high death mite rate in hives after one application of a *Bacillus thuringiensis* product. Later, other studies described a low incidence of mites in colonies when lactobacilli (Audisio et al., 2015) and a *B. subtilis* subsp. subtilis Mori2 (Sabaté et al., 2012) product were administered. The metabolic pathway by which CFS 1 causes *Varroa* mortality through the bee remains unknown. Although our obtained values cannot be compared to other mite control products, it could be promising as a natural alternative considering its positive impact on soluble protein in bees' abdomen. Our results support the potential use of symbiont-derived bee products to improve bee health. Commensal microbiota enhance immunity improving bee health through mechanisms that remain partially unknown (Crotti et al., 2013). Thus it is of paramount importance to address this lack of information in order to understand bee health (Caccia et al., 2016; Kwong et al., 2017). Consequently, there is a current need for more research in this area in order to explore the molecular mechanisms involved in this symbiotic relationship.

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