Use of a plant extract (ApiSave™), as a natural and novel alternative to synthetic chemicals, to control honeybee diseases in North America.

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ABSTRACT

In North America, honeybees are affected by several diseases including European Foulbrood (EFB) and viruses. To address this problem, beekeepers often opt for antibiotics and chemical treatments which in time contribute to the development of resistance to the treatment compounds by the affecting organisms and the presence of chemical residues in bee products. This situation has lead beekeepers and the beekeeping industry to try alternative solutions with natural products. In our research projects, we tried a natural and novel plant extract (ApiSave™), under different formats, to control bacterial and viral diseases affecting honeybee colonies. First, we reared honeybee larvae in the lab with 1% (v/v) and 5% (v/v) ApiSave™ and *Melissococcus plutonius* cells in royal jelly diets for 6 days. The resulting survival rate was greater or equal to 75% up to 4 days before declining, suggesting a potential for ApiSave™ to control EFB. The investigation of the virus load in the reared larvae showed the presence of all tested viruses (ABPV, BQCV, DWVA, DWVB, IAPV, KBV, LSV, and SBV) in all treatments but DWVB was the lowest in 5% (v/v) ApiSave™ treatment, Treatment 1, and Treatment 2, suggesting a possible inhibitory activity of the treatments against the virus. When the plant extract was used as treatment against *M. plutonius* in EFB infected colonies, the treatment with 15% (w/w) ApiSave™ suggested that the plant extract was able to control the disease in 3 colonies out of seven after loosing 3 colonies due the advanced state of the disease before treatment. This study demonstrated that at the right concentrations, the novel plant extract has the potential of controlling EFB and DWVB virus in affected honeybee colonies.

INTRODUCTION

European foulbrood (EFB) is one the several diseases affecting honeybee (*Apis mellifera*) larvae. Its causing agent, *Melissococcus plutonius*, a gram-positive bacterium which infects developing larvae through feeding are transferred to the larvae with contaminated food. It multiplies in the midgut of the larvae and competes for nutrients with the host, resulting in the death of the larvae by starvation before they pupate (Forsgren 2010). In Canada, antibiotics are used to treat the disease as prophylactic and curative treatments, leading to the development of resistance by the pathogen and potential contamination of honey. This has prompted the Canadian government to impose a veterinary prescription before an antibiotic can be used to treatment honeybee diseases in 2018. Since then, beekeepers have turned to alternative options such as the use of natural products to combat honeybee diseases (Hashish et al. 2016; Hassona, 2017; Khattiaby et al., 2011; Khan et al. 2019; Kim et al., 2018). Viruses such as the acute bee paralysis virus (ABPV), Kashmir bee virus (KBV) and Israeli acute paralysis virus (IAPV), mostly transmitted by Varroa destructor, also cause devastating diseases in the beekeeping industry (de Miranda et al., 2010a). The present study aims to determine the effect a plant extract (Api-Save™) on *M. plutonius*-infected honeybee larvae in-vitro, the microbial and viral loads in the larvae after the in-vitro larvae rearing, and whether Api-Save™ can control EFB affected colonies in the field.

MATERIALS AND METHODS

- **In-vitro rearing:** The honeybee larvae rearing was based on work by Schmehl et al 2016. Honeybee larvae aged 90 h were transported to the lab, grafted, and fed three diets consisting of sterile water, sugars, yeast, royal jelly, and different concentrations of Api-Save™ for five days post grafting. Each day, larvae were monitored for mortality before feeding.
- **Bacteria and virus loads**: Larvae that survived the rearing were collected, crushed in PBS, serially plated on M110 agar for *M. plutonius* quantification followed by PCR confirmation and virus quantification by Real-time PCR.
- ApiSave™ **treatment in the field:** For the field experiment, 3 groups (1 control and 2 treated) of hives were treated with a mixture of lyophilized Api-Save™ and in icing sugar at different concentrations of ApiSave™ (2017 Manitoba Agriculture's Recommendations for Administering Antibiotics and Acaricides to Honey Bees) over 2 weeks.

Table 1. Larval feeding time and diet amount, based on Schmehl et al 2016.

Time after grafting	Diet	Amount of total diet (µl)
Day 0 (grafting day)	A	20
Day 1	No feeding	0
Day 2	A	20
Day 3	B	30
Day 4	C	40
Day 5	C	50

RESULTS AND DISCUSSION

Honeybee larvae *in-vitro* rearing

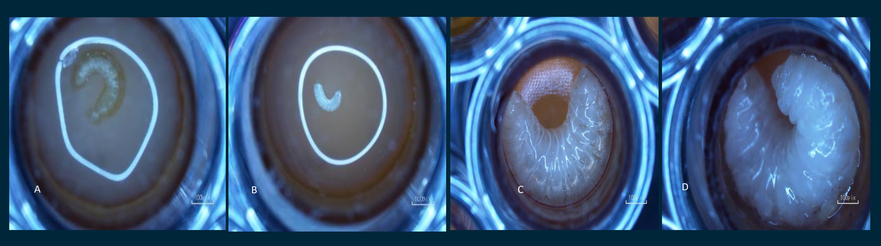

Figure 1. Honeybee larvae rearing: dead larvae (A and B), Live larvae (B), disintegrated (C and D)

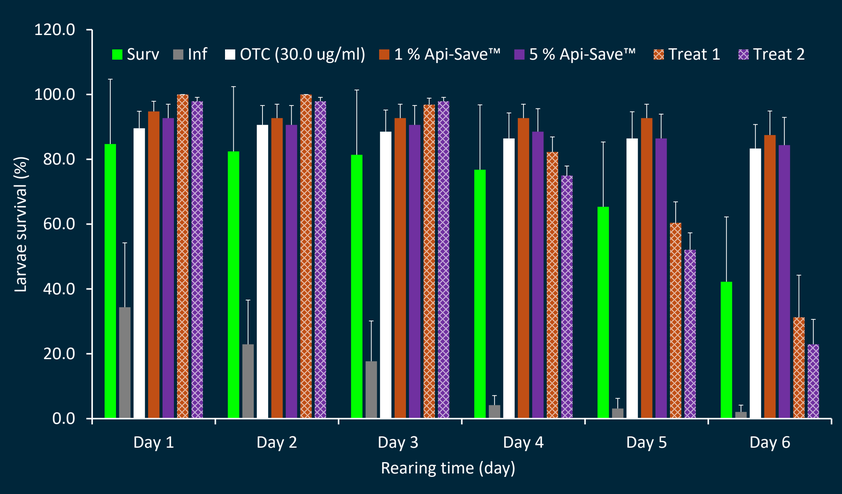

Figure 2. In-vitro rearing of *M. plutonius* infected honeybee larvae treated with liquid ApiSave™

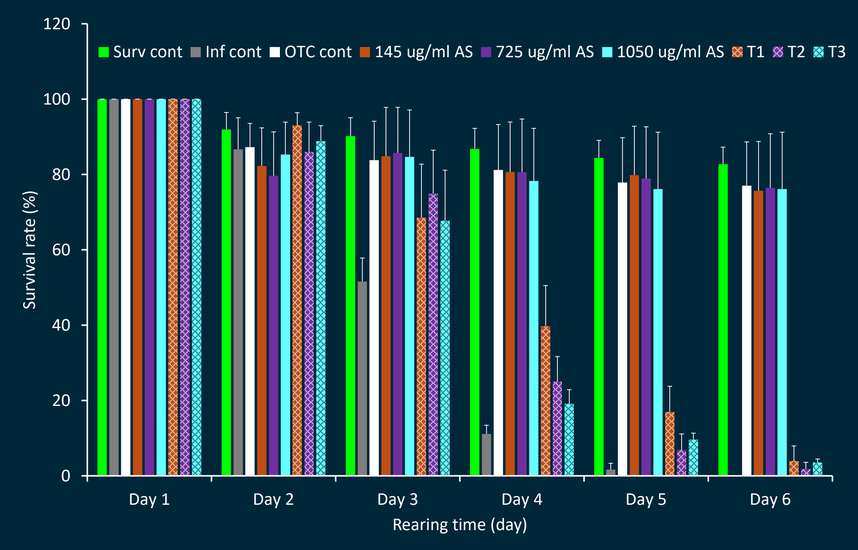

Figure 3. In-vitro rearing of *M. plutonius* infected honeybee larvae treated with lyophilized ApiSave™

Bacteria and virus loads

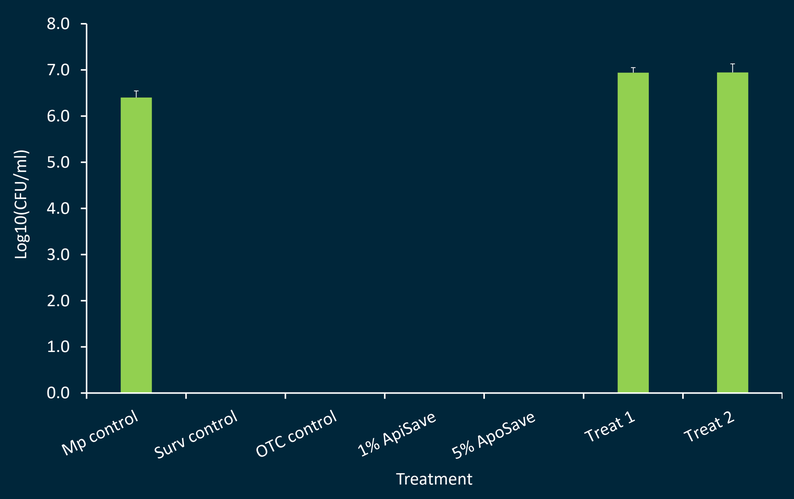

Figure 4. *M. plutonius* load in reared honeybee larvae

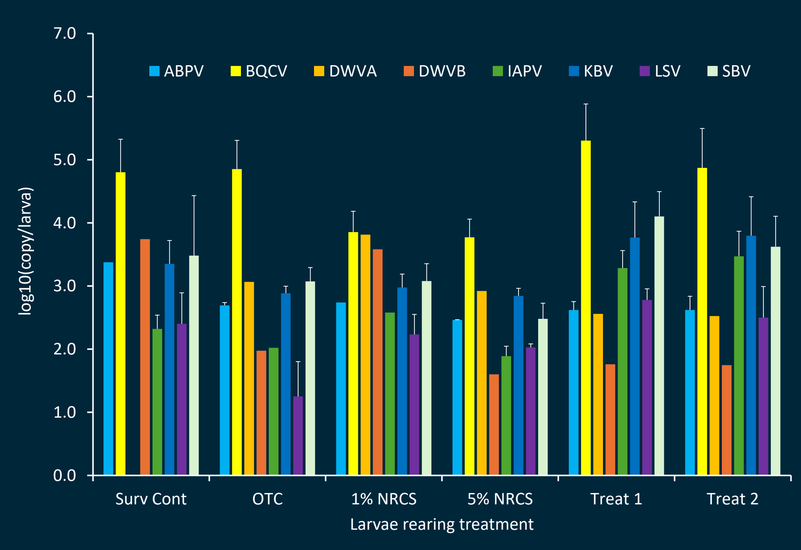

Figure 5. Assessment of colony health status before and after the last treatment with ApiSave™

Honeybee hives treatment in the field

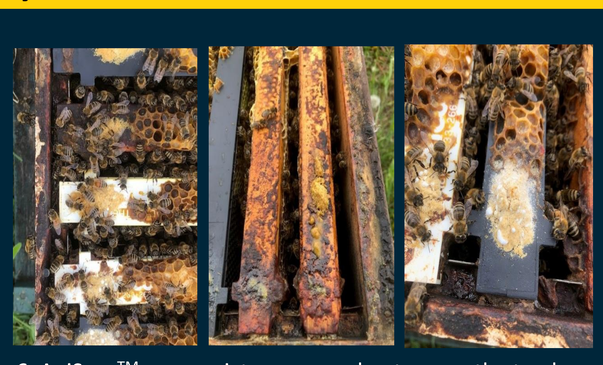

Figure 6. ApiSave™-sugar mixtures spread out across the top bars of the frames.

Table 2. Assessment of colony health status before and after the last treatment with ApiSave™

Colony #	# frames w/ bees out of 9)		% frame with bees		Visual symptoms		Disease Severity		Queen status	
	Before	After	Before	After	Before	After	Before	After	Before	After
3	6	6	67	67	>100	1-10	Severe	Low	Queen right	Queen right
6	1	Dead	11	0	10-100	Dead	Severe	Dead	Queen right	Dead
7	1	Dead	11	0	10-100	Dead	Severe	Dead	Eggs, no queen	Dead
8	4	4	44	44	10-100	1-10	Moderate	Low	No eggs, no queen	Queen right
10	2	Dead	22	0	>100	Dead	Severe	Dead	Queen right	Dead
11	1		11	11	10-100	None	Moderate	None	Eggs	Queen right
12	6		67	56	10-100	10-100	Not EFB	Moderate	Eggs	Eggs

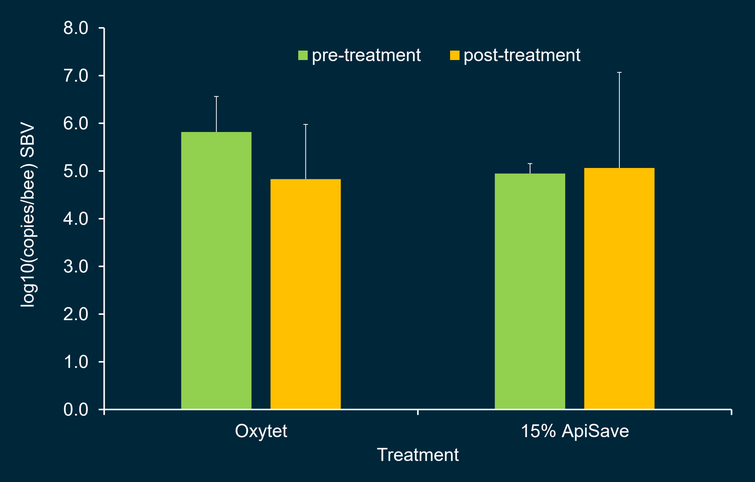

Figure 7. Assessment of colony health status before and after the last treatment with ApiSave™

CONCLUSION

- *M. plutonius*-infected honeybee larvae treated with ApiSave™ (1 and 5%; 0.05, 0.07, and 0.1%) during in-vitro rearing provided survival rate of greater from 67 to 75% close to survival control but way greater than infected control (≤ 4.2%) on day 4.
- The analysis of bacteria and virus loads indicated that *M. plutonius* had a higher content in larvae with 1 and 5% ApiSave™ that the fresh bacteria culture used probably because of the sugar content in the royal jelly from the diet, compared to the growth medium (M110 agar). Although there was no drastic impact of ApiSave™ on the viruses, the low content of DWVB in 5% ApiSave™ treatment may suggest an inhibitory effect on DWVB.
- After treatment (15% ApiSave™ in icing sugar) of *M. plutonius-infected* colonies in the field, the pathogen was still detected but it was able to control the disease in 3 colonies out of 6. At the conditions of the experiment, ApiSave™ could not control sacbrood virus (SBV).

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