Advances in the study of the toxicity of propolis alcoholic extracts on *Varroa destructor* and *Apis mellifera*

Natalia Damiani [a,b], Matías D. Maggi [a,b], Liesel B. Gende [a,b], Claudia Faverin [c], Victor Crouzat [a], Martín J. Eguaras [a,b], Jorge A. Marcangeli [a]

[a] Laboratorio de Artrópodos, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, Mar del Plata 7600, Argentina.
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
[c] Grupo Sistemas de Producción, Departamento de Producción Animal, EEA INTA Balcarce, cc 276, Balcarce 7620, Argentina

E-mail: ndamiani@mdp.edu.ar

INTRODUCTION

The parasitic mite, *Varroa destructor* affect the honey bee, *Apis mellifera*, causing great economic losses to the beekeeping industry. Efforts to control this pest have focused on the evaluation of synthetic acaricides but the development of acaricide resistance in mites populations and the contamination of hive products have evoked the researches on natural acaricides. A natural product that can be taken into account is propolis. Propolis is based on resins collected by bees from plants. Its biological activity is given by its high resin content, mainly phenolic compounds. Nevertheless, antecedents on the use of propolis as acaricide or insecticide are very limited. The aim of this work was to evaluate the toxicity of a propolis alcoholic extract on *V. destructor* and *A. mellifera* by means of different ways of administration.

MATERIALS AND METHODS

Propolis sample

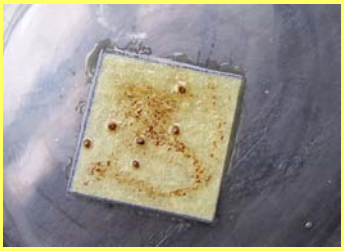
The propolis sample obtained from a apiary placed in Camet, Mar del Plata, Buenos Aires province, Argentina (37°53'S; 57°36'W) was organoleptic and physic-chemically characterized in the Agroindustries Laboratory, Famaillá Agricultural Experimental Station, National Institute of Agricultural Technology, Tucumán province. The organoleptic properties assessed were: appearance, consistency, visible impurities, aroma, flavour and colour. The physicochemical properties analyzed were: content of water, ash and wax, mechanical impurities, total resins, total phenols and total flavonoids (expressed as quercetine dihidrate) according to the protocol of IRAM-INTA norms (IRAM-INTA Norm 15935-1).

For all test, a propolis soft extract was obtained according to Cunha *et al.* 2004.

All treatments were carried out at room temperature (22-24°C) with worker of A. mellifera and adult female V. destructor collect from bees brood. All treated experimental animals were incubated at 28°C and 60% R.H.

All statistical analysis using different procedures from SAS sowfware (SAS Institute 2007).

Topical application



The soft extract was dissolved in 55% ethanol. The treatment concentrations were 1.25, 2.5, 5, 7.5 and 10% (w/v). A methodology adapted from Garedew *et al.* (2002) was used, where 200 µl of a specified concentration of propolis solution were applied on six mites placed on a piece of filter paper. Each treatment was stopped removing the mites from the filter paper, after they had remained in contact with the solution during 15, 30, 45, 60, 75 or 90 s. Five replicates for experimental unit and controls treating mites with 55% ethanol during the different contact times were done. The activity of mites was observed at 10 min, 30 min, 60 min and each 1 hour for the next seven hours, after the beginning of each treatment. Each individual mite was classified as mobile or inactive. If a mite remained inactive after 8 h from the beginning of treatments, it was considered dead.

Spraying application

Petri dishes arranged with absorbent filter paper on the inner bottom and an extra lid of metallic mesh, were used. Ten adult female mites and ten adult worker bees were placed in every modified dish. Once mites were attached at the body of the bees, 3.4 ml of 10% propolis solution were sprayed on bees throughout the metallic mesh by a hand sprayer. A device with candy and water was placed inside each bioassay unit as food for the bees. Petri dishes with bees and mites were sprayed with alcohol 55% as controls. Five replicates for each experimental group were made. Dead bees and dead mites were assessed at 24, 48, and 72 h.

Oral administration

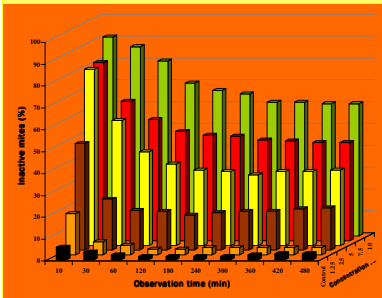
An average of 340.8 ± 59 adult worker bees coming from colonies with high prevalence of *V. destructor* was included in individual cages according to Maggi *et al* (2009). Propolis solution was administered in 10 mL of 2:1 alcoholic syrup as food for bees. Concentrations tested were: 5, 10, 15 and 20 %. Each treatment was replicated five times. Bees only feeding with 10 mL of 2:1 alcoholic syrup were controls. All cages were maintained with a synthetic queen pheromone. After 24 h, all bees were fed only with 2:1 syrup, depending on demand. Dead bees and mites were recorded at 24, 48 and 72 h. The final number of mites and bees from each cage was registered.

RESULTS

Propolis sample

Aspect	opaque and shiny irregular fragments
Consistence	soft
Visible impurities	remains of plants and wood
Aroma	aromatic resinous
Flavor	sweet
Color	greenish-yellowish brown
Water content	0.82 %
Wax content	15.06 %
Ash content	3.65 %
Mechanical impurities	5.89 %
Total resins	77.45 %
Total phenols	21.74 %
Total flavonoids	9.18 %

Topical application



Topical treatments with the propolis solution showed a narcosis effect on *V. destructor*. This effect was noticed when the mites that remained inactive during the firsts hours after the beginning of treatments, then recovered its activity, regardless of the treatment concentration and the contact time with the solution. Although some mites were able to recover from the narcosis, others could not recuperate fully. The mites that did not get back its activity during the firsts 8 h after treatments were considered dead. The acaricide action incremented as increasing concentrations of propolis extracts. Contact times of 30 seconds were enough for evoking narcosis and mortality effects.

Spraying application

When the effect of treatment of *Varroa* infested bees with 10% propolis solution by spraying application method was evaluated, the proportion of dead mites was significantly different from control treatment at 24 h, 48 h and 72 h. In contrast, the proportion of dead bees after treatment was not significantly different from the control treatment (Table 1).

Table 1. Mean percentage of dead mites + S.E and dead bees + S.E at 24, 48 and 72 h after treatment of infested bees with the 10% propolis solution by spraying application method.

	Observation times		
	24 h	48 h	72 h
Mites			
Control	2 (1.98) a	6 (3.359) a	8 (3.837) a
Treated	48 (7.065) b	68 (6.597) b	78 (5.858) b
Bees			
Control	6 (3.359) a	8 (3.837) a	10 (4.243) a
Treated	6 (3.359) a	8 (3.837) a	10 (4.243) a

* 10 bees and 10 mites per experimental unit. Standard error: S.E. Means with different letters indicate statistically significant differences ($P < 0.01$) between treatments within groups.

Oral administration

Table 2. Mean percentage of dead mites + S.E at 24 h, 48 h and 72 h after treatment of naturally infested bees in cages fed with different concentrations of propolis solution by the oral administration method

	Bees			Mites		
	24 h	48 h	72 h	24 h	48 h	72 h
control	7.87 (1.58) ac	9.01 (2.01) a	9.30 (1.90) a	6.35 (3.72)	6.35 (3.72)	6.35 (3.72)
5 %	4.78 (1.80) ab	7.71 (2.82) ab	8.76 (3.01) ab	1.54 (1.54)	1.54 (1.54)	1.54 (1.54)
10 %	2.45 (0.66) b	2.94 (0.72) b	3.35 (0.86) b	0 (0.00)	0 (0.00)	0 (0.00)
15 %	3.16 (0.36) b	5.75 (0.62) ab	6.60 (0.97) ab	1.82 (1.82)	4.32 (2.70)	4.32 (2.70)
20 %	12.02 (1.44) c	22.60 (2.12)c	25.23 (2.80) c	4.58 (2.80)	6.86 (4.21)	12.97 (7.25)

* Standard error: S.E. Means followed by the same letter are not significantly different ($P > 0.05$). Prevalence of *V. destructor* in the cages: 4.49 ± 2.

When propolis extracts were orally administered in cages with infested bees the proportion of dead mites at 24, 48 and 72 h was not significantly different among all propolis concentrations (Table 2). However, the effect of the different propolis concentrations was significantly different for the bees along the observed period. The proportion of dead bees at each observation time was not different among the firsts three propolis concentrations administered but in the treatment with the highest concentration this proportion was significantly different from the control and from the others treatment concentrations at 48 and 72 h.

CONCLUSIONS

- The organoleptic and physicochemical properties of the propolis sample were according with data registered for other propolis samples from Pampean region (Bedascarrasbure *et al.*, 2006). Due to the elevated content of biologically active components such as phenols and flavonoids, propolis collected from this zone have the best quality in Argentina.
- Narcosis and acaricide activity of propolis extracts was found when *Varroa* mites were topically treated.
- Spraying treatment of infested bees were innocuous for bees but mites resulted highly sensitive to propolis solutions.
- Oral administration of propolis in syrup resulted not toxic on mites even at harmful concentrations for bees.

The propolis extracts from our geographical zone could be incorporated into honey bee colonies by spraying, although still remain to adjust the potential doses and concentrations to be administered and the mechanism of action of the propolis on mites. Although it is possible that the propolis, by itself, is not useful to parasitic mite control, we could not exclude a possible indirect effect of the propolis due to the stimulation of immune system of the bees when it is orally applied. Further investigations are required to get a full knowledge about the effects of propolis alcoholic extracts on *V. destructor*. The variability in the propolis chemical composition according to the phytogeographical origin and the feasible ways of administration of the extracts in hives, are the main factors to be taken into account in planning future works, by incorporating propolis extracts into a Management Integrated Program that allow reducing the amount of synthetic acaricides in the hives.

Research in progress

A. Full mini-hive
B. Open mini-hive with bees on 8 combs and 2 feeders.
C. One comb with bees being manually sprayed with propolis solution.
D. Treatment with coumaphos at the end of assay.

Each comb from the mini-hive was sprayed with a handed sprayer containing propolis solution (10% of propolis extract in 2:1 alcoholic syrup). That applications were repeated 3 times during a month.

Preliminary results showed a differential efficacy from control treatment.

For more information contact the authors

Acknowledgments: to IZS (Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Roma) for to make possible the presentation of the present poster

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