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ORIGINAL PAPER

Bioactivity of propolis from different geographical origins on *Varroa destructor* (Acari: Varroidae)

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Abstract *Varroa destructor* is an ectoparasitic mite that affects colonies of honey bee *Apis mellifera* worldwide. In the last years, substances of botanical origin have emerged as natural alternative acaricides to diminish the population levels of the mite. In the present work, the bioactivity of propolis from different geographical locations of Pampean region from Argentina on *V. destructor* was evaluated. Fourteen propolis samples were organoleptic and physicochemically characterized and, by means topical applications, their activity was tested on mites. All propolis had a homogeneous composition and the bioactivity levels against mites were comparable among the different propolis samples. The percentage of mites killed by the treatments ranged between 60.5% and 90% after 30 s of exposure. Thus, *V. destructor* was highly susceptible to propolis. Moreover, the mites remained anesthetized during the first hours after topical treatment. The results suggest that propolis from Argentinean pampas could be incorporated in honey bee colonies as acaricidal treatment by spraying.

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Introduction

The colonies of the honey bee *Apis mellifera* are affected by a severe parasitosis evoked by the ectoparasite mite *Varroa destructor* (Anderson and Trueman 2000). To avoid its death, colonies must be treated against the disease. Synthetic acaricides have been the traditional way of control during the last years but resistant mites and residues in honey bee products have increased worldwide (Bogdanov et al. 1998; Milani 1999; Wallner 1999) even in Argentina (Maggi et al. 2009). For this reason, substances of botanical origin have emerged as natural alternative acaricides to diminish the population levels of *V. destructor*. For the control and prevention of honey bee pathologies, there is a recent inclination toward botanical substances. Several essential oils and botanical extract have been used with variable success against American foulbrood disease (Gende et al. 2009), chalkbrood (Dellacasa et al. 2003), *Nosema* infections (Pohorecka 2004) and varroosis (Damiani et al. 2009). Few studies have been made about propolis and honey bee diseases despite its botanical origin (Antúnez et al. 2008; Garedew et al. 2002; Gende et al. 2007).

Propolis consists of a mixture of resins and waxes that are collected by bees from various plant species, particularly of flowers and leaf buds. It is difficult to observe bees in their foraging task, therefore, the precise source from where the resins are obtained, is usually unknown. It has been observed bees scraping the protective resins from the flowers and leaf buds with their mandibles and then carrying them into the hive as pellets on their hind legs.

In the process of gathering and elaborating of propolis, the resins are mixed with a little saliva and other secretions of bees and wax (Burdock 1998). These resins are used by bees to cover the cavities inside the nest and brood combs, to repair combs, to seal small cracks in the hive, to reduce the size of the hive entrance, to seal large dead animals within the hive and, perhaps most importantly, mixing small quantities of propolis with wax to close the brood cells (Bankova et al. 2000). Thus, propolis provides antibacterial and antifungal effects to the colony's environment that strengthens protection against diseases.

Propolis should not only be free of pollutants, but also the percentage of inert substances in relation to its biological action, such as wax, insoluble particles and ash, must be recorded. However, the most important feature is its level of biological activity, which is essential to characterize the abundance of biologically active components present in a propolis sample. Because bees collect propolis from different plants depending on the geographic location and specificity of the local flora, a significant variation in the chemical composition of propolis complicate the quantification of their bioactive compounds. It is commonly believed that a sample of propolis is high quality if it contains a high percentage of flavonoids (Bonvehi and Coll 1994; Park et al. 1998). A literature review about the biological action of the components showed that this statement is, in some cases erroneous. Antibacterial substances with no-phenolic origin have been isolated from propolis collected in Brazil (Bankova et al. 1996). Thus, the biological activity of propolis is given by its high content of resins, primarily (but not exclusively) phenolic compounds, predominantly flavonoids (Bankova et al. 1983). The specific flora that is accessible for bees and geographical and climate features of the area where these resins have been collected by bees, determine a very variable composition of the propolis (Bankova 2005; Bedascarrasbure et al. 2006).

Numerous studies have proven the versatile pharmacological activity of propolis: bacteriostatic, bactericidal, antifungal, antiviral, cytotoxic, anti-inflammatory, antioxidant, antitumor, among others (Banskota et al. 2001; Marcucci 1995). In recent years, propolis has caught the attention of many researchers because of the multiple possibilities for use in human and veterinary medicine where its biological activity has been demonstrated against several parasites (Freitas et al. 2006; Higashi and de Castro 1994; Topalkara et al. 2007), herpes virus (Huleihel and Isanu 2002), HIV (Harish et al. 1997), and cancer (Oršolić et al. 2006). However, antecedents about acaricide and insecticide effects of propolis are very limited. Some researchers have demonstrated that the treatment with propolis decreases the larval growth and duration of the pupal metamorphosis, and are toxic to different developmental stages of the wax moth *Galleria mellonella* (Garedew et al. 2004; Johnson et al. 1994). Garedew et al. (2002) showed that *V. destructor* is sensitive to propolis solutions applied topically on it. The aims of the present work were to characterize propolis from different geographical locations of Pampean region from Argentina and to evaluate the bioactivity of the propolis extracts on *V. destructor*.

Experimental

Collection and analyses of propolis samples

The propolis samples were obtained making contact with beekeepers whose beehives are placed in different zones of Pampean region. In Table 1, the geographical location where each sample was collected is detailed.

Upon receipt, each sample was inspected in order to find rests of bees, wood, plant, pupa of moth, among other. The major visible impurities were removed from the samples by hand. Each sample was weighed, frozen, ground with a mortar, and then stored at 4°C until use. The propolis were organoleptic and physicochemically characterized in the Agroindustries Laboratory, Famaillá Agricultural Experimental Station, National Institute of Agricultural Technology, Tucumán province. Appearance, consistency, visible impurities, aroma, flavor, and color were the organoleptic properties assessed. The physicochemical characterization was made in relation to the contents of water, ash and wax; mechanical impurities, total resins, total phenols, and total flavonoids (expressed as quercetine dihydrate)

Table 1 Geographical origin of propolis samples

Sample Nº	Geographical origin
1	Balcarce (37º53′ S; 58º15′ W), Buenos Aires
2	Tres Arroyos (28º24′ S; 60º18′ W), Buenos Aires
3	Mar del Plata (37º53′ S; 57º37′ W), Buenos Aires
4	Coronel Vidal (37º26′ S; 57º43′ W), Buenos Aires
5	Lobos (35º11′ S; 59º06′ W), Buenos Aires
6	Villa Paranacito (33º44′ S; 58º40′ W), Entre Ríos
7	Capilla del señor, Campana (34º10′ S; 58º56′ W), Buenos Aires
8	Campana (34º07′ S; 58º52′ W), Buenos Aires
9	Islas del Ibicuy (33º39′ S; 58º49′ W), Entre Ríos
10	Berisso (34º52′ S; 57º52′ W), Buenos Aires
11	La Plata (34º54′ S; 57º56′ W), Buenos Aires
12	Olmos (34º45′ S; 58º34′ W), Buenos Aires
13	Manuel B. Gonnet (34º55′ S; 58º02′ W), Buenos Aires
14	Escobar (34º54′ S; 57º56′ W), Buenos Aires

according to the protocol of IRAM-INTA norms (IRAM-INTA Norms 15935-1, 2008).

Extraction

For the bioassay, each single soft extract of propolis was obtained from a suspension elaborated from pulverized propolis and ethanol 70% at 1:9 (*w*/*v*) ratio according to Cunha et al. (2004). Thus, the suspension was extracted at 60°C for 2 h in constant shaking; then it was cooled at room temperature and filtered by suction. After filtrated, the solution free of wax and impurities was evaporated at 40°C up to obtained a soft extract. The humidity content of the soft extract was determined according to IRAM-INTA Norms 15935-1 (2008). The wet weight/dry weight ratio was used to prepare solutions with different concentrations of each single propolis.

Experimental animals

Adult female mites of *V. destructor* were obtained from *A. mellifera* colonies placed in an experimental apiary of the National University of Mar del Plata, Mar del Plata, Argentina (38º10′06″ S; 57º38′10″ W). All colonies had been left untreated for *Varroa* for the preceding 12-24 months. Parasitized brood combs were carried to the laboratory, and their healthy capped cells were opened and inspected in search of mites. To avoid starvation, the mites were kept in Petri dishes on bee larvae or pupae during the collection process. The mites that appeared recently

Table 2 Organoleptic characteristics of propolis samples

Organoleptic characteristics		Propolis sample Nº													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Appearance	Granulated	X	X	X		X		X	X					X	
	Bright irregular pieces	X		X	X		X	X	X		X			X	
	Opaque irregular pieces				X	X				X	X		X	X	X
	Opaque irregular mass											X			
Consistency	Very soft												X		X
	Soft	X	X			X		X		X	X	X			
	Hard			X	X		X		X					X	
Visible impurities	Wood shavings	X		X	X				X		X				
	Remains of bees	X	X	X	X	X	X	X	X		X		X	X	X
	Bee wax	X	X	X	X		X	X		X			X	X	X
	Plant debris	X		X	X	X	X	X			X		X	X	X
	Moth cocoons	X		X			X			X			X		
	Remains of mesh		X												
	Scraps of paper/cardboard		X	X											
Aroma	Soft resinous		X						X		X			X	
	Resinous							X		X		X	X		
	Aromatic resinous	X				X	X								X
	Very aromatic resinous			X	X										
Flavor	Insipid								X	X		X	X	X	X
	Bitter				X	X		X						X	
	Sweet		X												X
	Spicy	X					X				X				
Color	Dark brown					X					X	X	X	X	
	Light brown				X		X		X						X
	Greenish					X				X	X	X			X
	Yellowish		X		X		X		X	X					
	Chestnut	X	X	X				X		X				X	
	Orangish	X						X				X			

The number of the propolis samples corresponding to *1* Balcarce, *2* Tres Arroyos, *3* Mar del Plata, *4* Coronel Vidal, *5* Lobos, *6* Villa Paranacito, *7* Capilla del Señor, *8* Campana, *9* Islas del Ibicuy, *10* Berisso, *11* La Plata, *12* Olmos, *13* MB Gonnet, *14* Escobar

molted, weak or abnormal were discarded because they may have a differential response during trials. All treatments were carried out at room temperature (22-24°C). The treated experimental animals were incubated at 28±1°C and 60% R.H.

Topical application method

The soft extracts were dissolved in 55% ethanol. The treatment concentrations were 2.5%, 5%, 7.5%, and 10% (*w*/*v*). The topical applications on the mites were made using a methodology adapted from Garedew et al. (2002). For each trial, 200 μl of a specified concentration of propolis were applied on six mites placed on a piece of filter paper. Each treatment was stopped when mites were removing from the filter paper (3×3 cm), after they had remained in contact with the propolis during 30 s, minimum contact time required for that mites have a differential response (Damiani et al., unpublished data). Then, parasites were transferred to a clean Petri dish (90×15 mm). Five replicates for each experimental unit were done. Controls treating mites with 55% ethanol were used. All tests were carried out at room temperature (22°C) and treated mites were incubated at 28±1°C and 60% RH. The activity of mites was observed under dissecting microscope at 10, 30, 60 min; and each 1 h for the next 7 h after the beginning of each treatment. Each individual mite was classified as mobile or inactive; it was considered inactive when did not show any movement in legs o the rest of the body when a stimulus was applied (Milani 1995). If a mite remained inactive after 8 h from the beginning of treatments, it was considered dead.

The proportion of inactive mites in each period of observation and for each concentration of treatment of every propolis sample was calculated. The treatments toxicity on mites was evaluated using SigmaStat 3.1 software. The effect on the mites of the different concentrations, over the total period of observation, of each individual propolis sample was analyzed by two-way RM analysis of variance (ANOVA) with observation time as a repeated measure. For multiple comparisons, the Tukey's test was used. Comparisons between the effects of the propolis from different geographical origin were evaluated by two-way ANOVA (origin and concentration) followed by multiple comparisons of least squares means using the Tukey's test.

Results

Analyses of propolis samples

The organoleptic features of each propolis sample are showed in Table 2. The physicochemical properties of each propolis extract are given in Table 3. The propolis extracts showed an average value of 66.86% of resins, 20.28% of total phenols, and 6.84% of flavonoids.

UV spectrograms showed that the propolis extracts analyzed displayed a maximum absorbance range between 270 and 315 nm. The propolis from Manuel B. Gonnet, Tres Arroyos, Mar del Plata, Coronel Vidal, Campana, Islas del Ibicuy, Berisso, La Plata, Olmos and Escobar exhibited a main absorption peak at 292 nm; while in propolis from Balcarce, Lobos, Villa Paranacito and Capilla del Señor, it was at 294 nm.

Table 3 Physicochemical properties of propolis extracts

Propolis sample	Wax content	Mechanical impurities	Total resins	Total phenols	Total flavonoids
Balcarce	16.38	6.08	76.45	22.19	7.75
Tres Arroyos	36.88	3.22	59.55	17.56	6.27
Mar del Plata	15.06	5.89	77.45	21.74	6.89
Coronel Vidal	12.84	5.24	81.44	23.78	6.11
Lobos	13.92	8.93	75.42	19.52	6.52
Villa Paranacito	29.32	0.87	68.79	20.41	6.14
Capilla del Señor	19.85	0.69	78.79	23.12	6.54
Campana	24.54	1.48	73.38	22.70	8.92
Islas del Ibicuy	39.11	7.78	52.11	16.11	6.56
Berisso	38.62	3.12	57.58	17.45	6.47
La Plata	40.29	1.37	56.39	19.96	6.51
Olmos	62.90	4.41	32.60	10.67	3.34
MB Gonnet	27.04	5.71	66.21	23.08	7.14
Escobar	41.11	3.20	54.68	20.62	6.96

Values are expressed in percentage (%) and represent an average of three determinations

Table 4 Percentage of dead mites ± SE after 8 h from treatment with different concentrations of propolis from different geographical origin

Propolis sample	Treatment concentrations			
	2.5%	5%	7.5%	10%
Balcarce	16.7 (6.33) Ba	46.6 (6.94) BCb	52.1 (5.48) BCDb	69.3 (6.33) Ab
Tres Arroyos	35.6 (5.86) ABa	56.8 (4.9) ABCbc	51.6 (4.90) BCDab	74.0 (4.48) Ac
Mar del Plata	15.4 (4.30) Ba	30.3 (4.68) Cab	46.9 (4.68) BCbc	60.5 (4.68) Ac
Coronel Vidal	12.3 (4.68) Ba	41.5 (4.90) BCb	55.2 (4.30) BCDb	73.8 (4.15) Ac
Lobos	22.2 (6.78) Ba	33.3 (7.42) BCab	47.6 (6.27) BCb	72.2 (6.78) Ac
Villa Paranacito	56.7 (7.42) Aa	53.3 (7.42) ABCa	60.0 (7.42) BCDa	73.3 (7.42) Aa
Capilla del señor	38.9 (6.78) ABac	50.0 (7.42) BCb	69.4 (6.78) BCDbc	69.4 (6.78) Ab
Campana	56.7 (7.42) Aac	86.7 (7.42) Ab	76.7 (7.42) ABbc	90.0 (7.42) Ab
Islas del Ibicuy	13.3 (7.42) Ba	33.3 (7.42) BCab	50.0 (7.42) BCDb	86.7 (7.42) Ac
Berisso	16.7 (5.86) Ba	30.0 (6.94) Ca	40.0 (6.94) Ca	76.6 (6.94) Ab
La Plata	36.0 (6.33) ABa	52.8 (6.33) BCab	66.7 (5.86) BCDb	69.1 (5.86) Ab
Olmos	10 (7.40) Ba	33.3 (7.40) BCab	43.3 (7.40) BCb	56.7 (7.40) Ab
MB Gonnet	37.8 (4.68) ABa	60.3 (3.88) ABb	76.9 (5.48) ADb	75.4 (4.15) Ab
Escobar	15.3 (4.68) Ba	44.3 (4.48) BCb	55.2 (4.30) BCDbc	71.4 (4.15) Abc

Means with at least one letter in common are not significantly different ($p>0.05$). Uppercase letters compare among propolis from different origins within each treatment concentration. Lowercase letters compare among different treatment concentrations within each single propolis. *SE* standard error

Topical applications

The treatments with alcoholic extracts of propolis obtained from the Pampean region of Argentina showed mortality effects on the mite *V. destructor*. In Table 4, the results of the assessment of these effects of propolis of different geographic origin on mites are detailed. In general, a trend towards an increase in acaricide action as increased concentrations of the extracts was observed, except when the treatments were performed with the propolis extract of Villa Paranacito, where there was no difference between the concentrations tested ($p>0.05$). The average percentage of mites killed by the treatments with 10% propolis solutions was 72.74%, varying between 56.7% (Propolis from Olmos) and 90% (Propolis from Campana), but these mortality rates were not significantly different between all propolis tested ($p>0.05$).

In addition to mortality effects, treatments with propolis caused narcosis effects on *V. destructor*. This effect was evident when a high proportion of mites that remained in inactive state during the first hours after onset of treatments regained their activity. All propolis, regardless of geographical origin, narcotized to the mites in some degree during the treatments. In the treatments with higher concentrations (7.5% and 10%), a significant proportion of mites remained narcotized during the first 2 h after contact with propolis (all $p<0.05$), except in the propolis from Paranacito Villa and Campana where these concentrations caused high mortality on the mites from the beginning of treatments. After treatment with low concentrations, the mites were recovered from the narcosis during the first hour (all $p<0.05$). Not all mites were able to entirely recover from the narcosis. The mites that did not regain its activity during the first 8 h after treatment were considered dead. In the control group, the effects of narcosis and mortality were not observed (all $p>0.05$).

Discussion

The biological activity of propolis on various microorganisms has been demonstrated (Burdock 1998). Moreover, its effects have been tested on certain parasites showing amoebic, antigiardial and tripanosomal activity (Freitas et al. 2006; Higashi and de Castro 1994; Topalkara et al. 2007). Recent researches have suggested the potential action of propolis extracts in the treatment of bee diseases, such as American foulbrood (Antúnez et al. 2008; Gende et

Table 5 Mean values of physicochemical properties of propolis from this research and from bibliographical reference data

Propolis from	Wax content	Mechanical impurities	Total resins	Total phenols	Total flavonoids
Present research	28.11	3.96	66.86	20.38	6.84
Reference	24.54	6.07	64.96	21.59	9.18

Data obtained from Bedascarrasbure et al. (2006) of propolis from Argentinean Pampas. Values are expressed in percentage (%)

al. 2007), the largest moth *G. mellonella* (Garedew et al. 2004) and the parasitic mite *V. destructor* (Garedew et al. 2002).

When propolis extracts are made in ethanol at 70%, the most biologically active components are obtained (Cunha et al. 2004). The main bioactive compounds found in the resinous fraction of propolis, are only soluble in alcohol solutions (Medana et al. 2008). For this reason, the parasites of honey bees are not affected by propolis applied on the walls of the hive by worker bees.

Organoleptic and physicochemical properties identified in the propolis samples used in these trials were consistent with data recorded in other propolis samples from the Pampean region (Bedascarrasbure et al. 2006). Due to the high content of biologically active compounds such as phenols and flavonoids, the propolis collected from colonies of *A. mellifera* from this geographical region have the highest quality in Argentina.

Concerning the effect of propolis extracts on *V. destructor*, a study showed that these mites are highly susceptible to propolis solutions (Garedew et al. 2002). In this previous research, propolis was applied topically on mites and treatment with a 10% solution resulted in 100% mortality. The toxicity rates were independent of time of exposure with the extract, indicating a high toxicity even with small contact times. However, in the present study, the percentage of mites killed by the treatment with propolis collected from different areas of the Argentinean pampas ranged between 60.5% and 90% after 30 s of exposure. The controversy between the results obtained by the German researchers and those presented here could be due to the difference in the botanical origin of propolis samples that determines a variable composition in the phenolic fraction of them. Thus, we can suppose that the acaricidal activity of propolis extracts is given by the bioactive components present in this fraction. In our study, the different propolis samples behaved statistically similar among them when their effects against *V. destructor* were tested. On the other hand, the propolis sample tested in the research of Garedew et al. (2002) was not characterized physicochemically therefore its composition is unknown; thus, ours results can not be fully comparative with yours. The samples used in the present research came from the Pampean region of Argentina and the results from their physicochemical analyses coincided with those obtained by Bedascarrasbure and his colleagues (2006) on 67 propolis samples from this same region (Table 5). On this basis, these issues suggest that all propolis from the Pampean region are homogeneous in composition and the bioactivity levels against mites are comparable among the different propolis samples.

Furthermore, a narcotizing effect was observed after the mites came in contact with propolis. Mites remained anesthetized during the first hours after topical treatment. The same effect was observed by Garedew et al. (2002). When planning a control treatment for varroosis in honey bee colonies, the narcosis generated by applying a substance, would result in the permanence of the mites in inactive state on the floor of the hive. In this situation, the parasites removed from their host, are unlikely to recover quickly and return back to parasitize a bee. It is possible that during this period of time, the bees will take it out of the hives during routine cleaning activities. Thus, the narcosis would be an added effect of propolis treatment that contributes positively to reducing the infestation levels of mites in the colonies. The power of narcosis plus the lethal effect caused when mites remain in contact with the propolis, demonstrate the potential of propolis extracts in controlling *V. destructor* by this method of administration.

The results suggest that propolis extracts from this area could be incorporated in honey bee colonies by spraying. Despite these promising results, the concentration, doses and the mechanism of action of propolis on mites need still to be adjusted so to optimize the acaricide potential observed in these bioassays. Garedew et al. (2002) suggested that contact with propolis solutions could lead to a weakening of the mites' cuticle that could facilitate entry of the active compounds present in propolis. Moreover, the effect of propolis extracts on bees has been little investigated; only one report of Antúnez et al. (2008) showed that bees tolerated high concentrations of alcoholic extracts of propolis administered orally with syrup. In order to implement its use as an alternative control substance, further researches are required to obtain a full knowledge about the effects of propolis extracts on *V. destructor* and honey bees. The variable chemical composition according to phytogeographical origin, the effects on *A. mellifera* and the possible methods for extract administration in the hives, are the main factors to be taken into account in planning future work to incorporate propolis in a program of integrated management which reduces the amount of synthetic acaricides in the hives.

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References

Anderson D, Trueman J (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Exp Appl Acarol 24:165–189. doi:10.1023/A:1006456720416

Antúnez K, Harriet J, Gende L, Maggi M, Eguaras M, Zunino P (2008) Efficacy of natural propolis extract in the control of

American Foulbrood. Vet Microbiol 131:324–331. doi:10.1016/j.vetmic.2008.04.011
Bankova V (2005) Recent trends and important developments in propolis research. eCAM 2(1):29–32, 10.1093/ecam/neh059
Bankova VS, Popov SS, Marekov NL (1983) A study on flavonoids of propolis. J Nat Prod 46(4):471–474. doi:10.1021/np50028a007
Bankova V, Marcucci MC, Simova S, Nikolova N, Kujumgiev A, Popov S (1996) Antibacterial diterpenic acids from Brazilian propolis. Z Naturforsch C 51(5–6):277–280
Bankova VS, De Castro SL, Marcucci MC (2000) Propolis: recent advances in chemistry and plant origin. Apidologie 31:3–15. doi:10.1051/apido:2000102
Banskota HA, Tezuka Y, Kadota S (2001) Recent progress in pharmacological research of propolis. Phytother Res 15(7):561–571. doi:10.1002/ptr.1029
Bedascarrasbure E, Maldonado L, Fierro Morales W, Álvarez A (2006) Propóleos. Caracterización y normalización de propóleos argentinos. Revisión y actualización de composición y propiedades. Ediciones Magna Publicaciones, Tucumán
Bogdanov S, Kolchenmann V, Imdorf A (1998) Acaricide residues in some bee products. J Apic Res 37:57–67
Bonvehi JS, Coll FV (1994) Phenolic composition of propolis from China and from South America. Z Naturforsch C 49:712–718
Burdock GA (1998) Review of the biological properties and toxicity of bee propolis (Propolis). Food Chem Toxicol 36:347–363. doi:10.1016/S0278-6915(97)00145-2
Cunha IBS, Sawaya ACHF, Caetano FM, Shimizu MT, Marcucci MC, Drezza FT, Povia GS, Carvalho PO (2004) Factors that influence the yield and composition of Brazilian propolis extracts. J Braz Chem Soc 15(6):964–970. doi:10.1590/S0103-50532004000600026
Damiani N, Gende L, Bailac P, Marcangeli J, Eguaras M (2009) Acaricidal and insecticidal activity of essential oils on *Varroa destructor* (Acari: Varroidae) and *Apis mellifera* (Hymenoptera: Apidae). Parasitol Res 106(1):145–152. doi:10.1007/s00436-009-1639-y
Dellacasa AD, Bailac PN, Ponzi MI, Ruffinengo SR, Eguaras MJ (2003) In vitro activity of essential oils against *Ascosphaera apis*. J Essent Oil Res 15(3):282–285
Freitas SF, Shinohara L, Sforcin JM, Guimarães S (2006) *In vitro* effects of propolis on *Giardia duodenalis* trophozoites. Phytomed 13:170–175. doi:10.1016/j.phymed.2004.07.008
Garedew A, Lamprecht I, Schmolz E, Schricker B (2002) The varroacidal action of propolis: a laboratory assay. Apidologie 33:41–50. doi:10.1051/apido:2001006
Garedew A, Schmolz E, Lamprecht I (2004) Effect of the bee glue (propolis) on the calorimetrically measured metabolic rate and metamorphosis of the greater wax moth *Galleria mellonella*. Thermochem Acta 413:63–72. doi:10.1016/j.tca.2003.10.014
Gende LB, Palazzetti M, Pannone O, Fritz R, Eguaras MJ, Saccares S, Dell'Aira E, Formato G (2007) Comparative field trials of *Paenibacillus larvae* control with antibiotics, essential oil of cinnamon and propolis. Proceedings of 40th Apimondia Congress
Gende LB, Maggi M, Damiani N, Fritz R, Eguaras MJ, Floris I (2009) Advances in the apiary control of the honeybee American foulbrood with Cinnamon (*Cinnamomun zeylanicum*) essential oil. B Insectol 62(1):93–97
Harish Z, Rubinstein A, Golodner M, Elmaliah M, Mizrachi Y (1997) Suppression of HIV-1 replication by propolis and its immunoregulatory effect. Drugs Exp Clin Res 23(2):89–96
Higashi KO, De Castro SL (1994) Propolis extracts are effective against *Trypanosoma cruzi* and have an impact on its interaction with host cells. J Ethnopharmacol 43:149–155. doi:10.1016/0378-8741(94)90012-4
Huleihel M, Isanu V (2002) Anti-herpes simplex virus effect of an aqueous extract of propolis. Isr Med Assoc J 4(11 Suppl):923–927
IRAM-INTA Norms 15935-1 (2008) NOA (Argentinean northwest) products. Propolis. Part 1: Propolis in natura. pp 25
Johnson KS, Eischen FA, Giannasi DE (1994) Chemical composition of North American bee propolis and biological activity towards larvae of greater wax moth (*Lepidoptera: Pyralidae*). J Chem Ecol 20(7):1783–1791. doi:10.1007/BF02059899
Maggi M, Ruffinengo S, Damiani N, Sardella N, Eguaras E (2009) First detection of *Varroa destructor* resistance to coumaphos in Argentina. Exp Appl Acarol 47(4):317–320. doi:10.1007/s10493-008-9216-0
Marcucci MC (1995) Propolis: chemical composition, biological properties and therapeutic activity. Apidologie 26:83–99. doi:10.1051/apido:19950202
Medana C, Carbone F, Aigotti R, Appendino G, Baiocchi C (2008) Selective analysis of phenolic compounds in propolis by HPLC-MS/MS. Phytochem Anal 19(1):32–39. doi:10.1002/pca.1010
Milani N (1995) The resistance of Varroa jacobsoni Oud to pyrethroids: a laboratory assay. Apidologie 26:415–429. doi:10.1051/apido:19950507
Milani N (1999) The resistance of *Varroa jacobsoni* Oud. to acaricides. Apidologie 30:229–234. doi:10.1051/apido:19990211
Oršolić N, Šaranović AB, Bašić I (2006) Direct and indirect mechanism(s) of antitumour activity of propolis and its polyphenolic compounds. Planta Med 72:20–27
Park YK, Koo MH, Abreu JAS, Ikegaki M, Cury JA, Rosalen PL (1998) Antimicrobial activity of propolis on oral microorganisms. Curr Microbiol 36:24–28. doi:10.1007/s002849900274
Pohorecka K (2004) Laboratory studies on the effect of standardized *Artemisia absinthium* L. extract on *Nosema apis* infection in the worker *Apis mellifera*. J Apicult Sci 48(2):131–136
Topalkara A, Vural A, Polat Z, Toker MI, Arici MK, Ozan F, Cetin A (2007) *In vitro* amoebicidal activity of propolis on *Acanthamoeba castellanii*. J Ocul Pharmacol Ther 23(1):40–45. doi:10.1089/jop.2006.0053
Wallner K (1999) Varroacides and their residues in bee products. Apidologie 30:235–248. doi:10.1051/apido:19990212