In vivo evaluation of antiparasitic activity of plant extracts on Nosema ceranae (Microsporidia)

Martín Porrini, Natalia Fernández, Paula Garrido, Liesel Gende, Sandra Medici, Martín Eguaras

Apidologie (2011) 42:700–707

DOI: 10.1007/s13592-011-0076-y

Original article

In vivo evaluation of antiparasitic activity of plant extracts on *Nosema ceranae* (Microsporidia)

Martín Pablo Porrini[1,2], Natalia Jorgelina Fernández[1,3], Paula Melisa Garrido[1,3], Liesel Brenda Gende[1,3], Sandra Karina Medici[1,3] Martín Javier Eguaras[1,3]

[1]Arthropods Laboratory, School of Exact and Natural Sciences, National University of Mar del Plata, Funes 3350 (7600) Mar del Plata, Argentina
[2]Agencia Nacional de Promoción Científica y Técnológica, Buenos Aires, Argentina
[3]Consejo Nacional de Investigaciónes Científicas y Técnicas, Buenos Aires, Argentina

Received 2 November 2010 – Revised 31 January 2011 – Accepted 17 February 2011

Abstract – This study evaluated the activity of plant extracts on *Nosema ceranae* development and their toxicity on the infected host *Apis mellifera*. Newly emerged bees were fed *ad libitum* with enriched syrups after individual infection. Diets consisted of ethanolic extracts obtained from *Artemisia absinthium, Allium sativum, Laurus nobilis*, and *Ilex paraguariensis* diluted in syrup at 1% and 10% concentrations. Examination of individual midgut homogenates on day 19 post-infection indicated that 1% concentration of *L. nobilis* extract significantly inhibited *N. ceranae* development. Absinth extract, previously reported as effective against *Nosema apis*, did not diminish the number of *N. ceranae* spores throughout the experiment. Ten percent concentrations showed high toxicity on infected bees, but also a significant activity diminishing parasitosis development in short periods. Syrups with the addition of extracts were consumed avidly as the control, even more in some cases. The present study constitutes the first report of antiparasitic activity *in vivo* of plant extracts against the Microsporidian *N. ceranae* and postulate natural substances as an alternative for antiparasitic treatment.

***Apis mellifera* / *Nosema ceranae* / plant extract / antiparasitic treatment**

1. INTRODUCTION

Among parasites affecting honeybees, nosemosis caused by *Nosema ceranae* is one of the most prevalent and pathogenic (Martín-Hernández et al. 2007; Higes et al. 2007; Paxton et al. 2007). This intracellular parasite has recently shifted hosts and seems to be the main microsporidian infection in *Apis mellifera* colonies (review in Fries 2010). These highly specialized fungi (Keeling and Fast 2002) are obligate intracellular parasites that spread among cells and hosts via spores.

The effective control of this disease is carried out by administering the antibiotic fumagillin (dicyclohexylammonium), which temporarily reduces parasitosis (Williams et al. 2008). This drug is the only one available for treatment and is no longer allowed in most EU member states because of possible residues in honey.

Research efforts to find effective and non-contaminant compounds against *N. ceranae* infection have been undertaken using different substances, such as essential oils, lisozime, thymol, and fitolexin resveratrol (Maistrello et al. 2008; Costa et al. 2010), as well as bacterial metabolites (Porrini et al. 2010).

Corresponding author: M.P. Porrini, mporrini@mdp.edu.ar
Manuscript editor: David Tarpy

Higher plants have been traditionally used as extracts to treat a number of infectious diseases, including those caused by bacteria, fungi, protozoa, and viruses (Soylu et al. 2005; Yoshida et al. 2005; Nejad and Deokule 2009). However, these substances are largely unexploited in conventional animal production and their effect on honeybees has been little studied (Pohorecka 2004a). The biological activity of extracts have been demonstrated against bee pathologies, such as American Foulbrood (the disease caused by the sporulated bacteria *Paenibacillus larvae*; Gende et al. 2008) and the parasitic mite *Varroa destructor* (Damiani 2010). Also, *Artemisia absinthium* extract has been tested against *Nosema apis* (Pohorecka 2004b), the first reported etiological agent of nosemosis in *A. mellifera*.

The aim of this work was to evaluate the bioactivity of plant extracts on *N. ceranae* development under laboratory conditions, with the particular goal of developing formulations for disease treatment, based on widely available plants.

2. MATERIALS AND METHODS

2.1 Bee collection and assays conditions

Experiments were carried out from November to December 2009 in the Arthropods Laboratory at the Universidad Nacional de Mar del Plata (Argentina). Newly emerged *A. mellifera* bees were obtained from a colony located in the experimental apiary J. J. Nágera coastal station, placed on route 11 km 32 (38° 10′06″ S, 57°38′10″ W) and kept under incubator conditions during the experiment (32°C±0.79; 60%± 3.3 HR).

2.2 *N. ceranae* strains

The *Nosema* spores used for inoculation were purified from a laboratory strain developed in confined workers. Spores were molecularly characterized following Martín-Hernández et al. (2007). Sequencing results were entered in the GenBank BLASTn, which yielded 98% homology with *N. ceranae* (accession number FJ425736).

2.3 Preparation of crude ethanolic extracts

Plant material was obtained as follows. Dry leaves of laurel, *Laurus nobilis* L. (Lauraceae), and absinth, *Artemisia absinthium* L. (Asteraceae); dry leaves and stems/trunks of yerba mate, *Ilex paraguariensis* St.-Hil. (Aquifoliaceae); and garlic cloves, *Allium sativum* L (Liliaceae) were collected in different geographic area from Argentina: laurel was obtained from Henderson (36°18′ S; 61°42′ W), Buenos Aires Province; absinth from Chapadmalal (38°10′06″ S; 57°38′10″ W) Buenos Aires Province; yerba mate and garlic were obtained as commercial samples packed in Misiones Province and Buenos Aires Province respectively. The samples of laurel and absinth were identified and deposited in the Faculty of Exact and Natural Sciences, National University of Mar del Plata herbarium.

After drying at 37°C for 24 h, the plant material was ground using mortar and pestle. Exposure to sunlight was avoided to prevent the loss of active components.

2.4 Extraction of selected plant material powder by maceration method

Five hundred milliliters of an 80% ethanol (extraction fluid) was mixed with 100 g of plant material. The mixtures were kept for 5 days in tightly sealed vessels at room temperature at 20°C, protected from sunlight, and mixed several times daily. This mixture was vacuum filtered and further extraction of the residue was repeated once until a clear colorless supernatant extraction liquid was obtained. The extracted liquid was subjected to evaporation in a water bath (40°C) to remove the ethanol until a semisolid state of extracted liquid was obtained. The semisolid extract produced was stored at 4°C until use (2 months for *L. nobilis, Y. paraguayensis*, and *A. sativum* while *A. absinthium* was used immediately after extraction).

2.5 Experimental design

The toxicity of the extracts on *A. mellifera* infected with *N. ceranae* and their antiparasitic effect was evaluated systematically. Ninety individuals per treatment (3 replicates of 30 bees), confined to experimental wooden cages with a plastic mesh (11×9×6 cm^3) participated in this assay. Workers were carefully removed from brood combs from the same colony

within 6 h, randomly confined, and supplied with sugar syrup 60% (*w*/*v*). Individual infection was achieved on day 3 (after emergence) using a technique modified from that published by Rinderer (1976). By means of this technique, bees are not anesthetized. Consumption of the inoculum occurs in a small container and is induced by previous starvation, thereby preventing oral structures manipulation to force intake and so minimizing the stress resulting from prolonged handling.

Bees were individually infected with 10 μL of solution (2.03×10^4 spores) and then supplied with one of the following diets: syrup enriched with 1% or 10% plant extract (garlic, laurel, absinth, or yerba mate) or control syrup. After inoculation, extracts dilutions were administered *ad libitum*, replacing the volumes on a daily basis until the end of the experiment. Extracts were supplied in cane sugar syrup so as to force bees to incorporate them in each feeding. In order to control unwanted infection due to food contamination, manipulation, or incidental ingestion of spores when the operculum is cut during bee emergence, 30 bees were confined to one cage. They were individually inoculated with 60% (*w*/*v*) sucrose syrup without spores (keeping the same diet after inoculation) and sacrificed at the end of the assay to quantify presence of spores on their midgut.

On days 7, 15, and 19 post-infection (p.i.), five bees per replicate were sacrificed in order to individually quantify the number of spores in the midgut (Cantwell 1970). Solutions evaporation was controlled to correct the consumed volumes.

2.6 Statistic analysis

Duncan multiple comparison test (α=0.05) was performed to test differences in spore loads. This test was used to determine significant differences among group means in the analysis of variance.

For each treatment, survival curves plotting number of live bees versus time were constructed using data from each cage. Gehan–Breslow nonparametric test was performed to determine whether survival curves were significantly different. Pairwise multiple comparisons were performed with Holm–Sidak method.

To verify the bees' dietary preference towards the experiment, average daily feed intake was compared using Kruskal–Wallis nonparametric test and comparisons with control treatment were performed by means of Dunn's method.

The statistical analysis of the results obtained was conducted applying α=0.05.

3. RESULTS

Significant differences in the number of Nosema spores occurred 7 days after artificial inoculation. At this time, 10% treatments of laurel ($6,6\times10^4$ spores on average) and yerba mate ($5,5\times10^4$ spores on average) showed spore counts significantly lower than control ($2,2\times10^6$ spores on average; p=0.037 and p=0.017, respectively).

On day 15 p.i. (Figure 1), garlic 1% treatment reached higher spore loads than those registered by yerba mate and laurel 1% (P=0.007 and P=0.003, respectively) but showed no differences with control treatment (P=0.084). Bees receiving 1% concentration treatments, except yerba mate, survived until the end of the experiment. Examination of individual midgut homogenates indicated that 1% concentration of *L. nobilis* extract significantly inhibited *N. ceranae* development on day 19, being the only treatment different from control at this time (P=0.017).

Bees not inoculated with spores did not develop a detectable level of disease at the end of the experiment. Survival data of these bees is not presented due to lack of replicates. Survival curves of bees fed with garlic, laurel, and absinth at 1% concentration did not differ significantly from control curve. Ten percent extract concentrations showed significantly lower median survival time compared to 1% groups for each herb (Figure 2, Table I).

At the end of the experiment, Kruskal–Wallis test (H=20.283, d.f.=8, P=0.009) and Dunn's multiple comparison procedure showed garlic 1% and laurel 1% as groups with higher daily intake than control treatment (P<0.05). The following average values of daily intake (microliter) were registered for every treatment at 1% and 10% concentration respectively: absinth 22.57 and 27.97; laurel 27.61 and

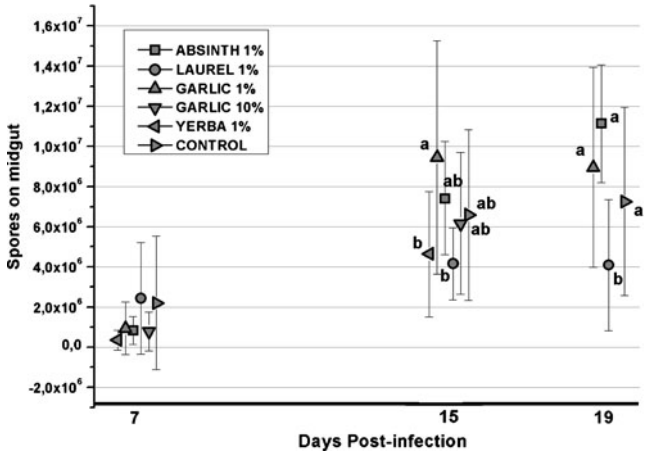

Figure 1. Parasitosis development for treatments alive after day 7 p.i. (absinth 1%, laurel 1%, garlic 1%, and 10%, yerba maté 1%, control treatment). Different *letters* indicate significant differences between treatments on the same day (Duncan test—approximate probabilities for post hoc tests, $P<0.05$). On day 7, no significant differences were detected between treatments.

22.95; garlic, 29.09 and 23.07; yerba mate, 23.30 and 21.18; control, 21.60.

It is worth noting that treated individuals showed no excessive droppings during confinement.

4. DISCUSSION

Our results demonstrate that *L. nobilis* extract is effective inhibiting in vivo development of *N. ceranae*. The time of action of this extract depended on its dosage. While 1% concentration significantly inhibited *N. ceranae* development after 19 days of treatment, 10% concentration seemed to be effective in a shorter period of time, decreasing parasitosis intensity but causing high mortality on bees. The antimicrobial activity of this herb was previously reported against another fungus species, such as *Candida albi-*

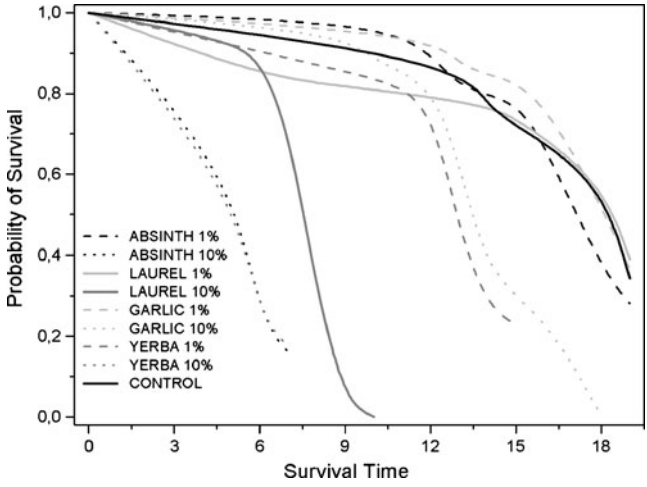

Figure 2. Survival curves from Gehan–Breslow test.

Table I. Mean survival time (days) ± standard error and *p* values from all pairwise multiple comparison among survival curves from Gehan–Breslow test.

		Absinth		Laurel		Garlic		Yerba maté		Control
Mean survival ± SE		1% **16.9**±0.40	10% **5.8**±0.1	1% **16.1**±0.71	10% **7.8**±0.18	1% **17.9**±0.37	10% **14.3**±0.44	1% **13.2**±0.25	10% **5.8**±0.10	**17.3**±0.36
p values	Absinth 1%		**1.02E−17**	5.75E−01	**8.40E−16**	6.46E−02	**1.48E−04**	**4.55E−06**	**3.98E−17**	5.14E−01
	Absinth 10%			**6.25E−13**	**1.29E−09**	**6.48E−17**	**1.88E−17**	**2.98E−18**	8.37E−01	**5.50E−18**
	Laurel 1%				**1.22E−05**	6.29E−02	7.52E−02	2.50E−02	**1.53E−12**	3.39E−01
	Laurel 10%					**6.60E−15**	**7.27E−15**	**3.19E−16**	**2.96E−09**	**3.80E−16**
	Garlic 1%						**3.32E−06**	**1.98E−07**	**2.38E−16**	2.87E−01
	Garlic 10%							2.10E−01	**7.21E−17**	**3.21E−05**
	Yerba maté 1%								**1.22E−17**	**4.07E−07**
	Yerba maté 10%									**2.20E−17**

Values under 0.001 (bold characters) indicate statistically significant difference among survivals'

cans and *Aspergillus niger*, showing higher inhibitory activity than the standard antifungal nystatin (Ertürk 2006). To the best of our knowledge, this study is the first report of anti-microsporidian activity of *L. nobilis* extract and, therefore, the ways of action remain unclear and further research is necessary to determine the identity of the active compounds. In any event, multiple constituents of plant extracts may be responsible of the therapeutic activity, being also possible synergic, additive, and antagonistic effects among the different compounds. Because of this, in future designs, the complete extract could be considered like an active "compound" (He 2000; Ong 2004).

A. absinthium extract, until now, the only herb extract tested against *Nosema* genus, was reported by Pohorecka (2004b) as inhibitor of *N. apis* after 17 days of treatment. However, our study found that both the highest concentration of absinth extract (10%) such as prolonged consumption of 1% concentration did not diminish the spore load of parasitic infection throughout the experiment. The differences between the two studies could be explained by a lower susceptibility of *N. ceranae* to the ethanolic extract of this herb or by factors that can influence on the chemical composition of the obtained substances, such as the extraction method (Tariq et al. 2009; Ahameethunisa and Hopper 2010). Pohorecka (op cit) administered a dilution of "standardized extract" without extraction method specifications, thus differences with these experiments may be attributable to this variable.

Despite the broad antiprotozoal activity of garlic (Mirelman et al. 1987; Ankri et al. 1997; Ankri and Mirelman 1999), ethanolic extracts from this plant tested at two concentrations did not influence significantly *N. ceranae* development along the experiment, even though 1% extract was consumed more than control syrup. The efficacy of chemical constituents of garlic chiefly depends on the mode of preparation and conservation of its extract. Allicin is one of the main active compounds of fresh garlic, but it is also one of the least stable (Block 1992; Lawson and Block 1997) with a half-life time which decreases significantly after contact with

ethanol (Lawson 1993). Therefore, future experiments involving the administration of non-ethanolic extracts and less storage time could provide different results.

The control of nosemosis disease in honeybee colonies requires treatments with an acceptable anti-microsporidial activity, without side effect on honeybees and minimizing toxic residues in wax and honey. Adamczyk et al. (2005) concluded that the presence of residues of natural substances in honey samples does not represent a sanitary risk, only that it may change the taste of the honey. As for bees, extracts tested in our experiment did not affect adversely the consumption of syrup. Furthermore, in some cases it was increased in comparison to control diet (i.e., garlic 1%, laurel 1%).

In our study, the spore load remaining after *L. nobilis* treatment (about four million) was significantly lower than other treatments and control, but mortality did not differ between them. These results are supported by previous studies (Mayack and Naug 2009; Porrini et al. 2011), which demonstrated that workers infected with *N. ceranae* survived just as well as uninfected or less-infected bees when fed *ad libitum*.

There is little information about the effect of plant extracts on honeybee health. Pohorecka (2004a), administering various standardized extracts, found no effect on midgut pH, no significant differences in pharyngeal glands degeneration during the experiment, and an increased fat body development (only for nettle extract). In the present study, extracts of garlic, absinth, and laurel at 1% concentration showed a median survival similar to control and, therefore, its toxic effect on young bees would be minimal. Given this low mortality, 1% concentration is recommended for long-term experiments.

Many vegetable extracts include some secondary products, such as polyphenols, which are toxic to insects (review in Berbehenn and Martin 1994). Honeybees usually avoid the contact with nectars that contains such secondary compounds (Adler 2000), but in our study, diet selection was not allowed. The high toxicity of yerba mate, inclusively at 1% concentration, may be explained by the presence of these (Rodríguez Vaquero et al. 2009) or similar substances. Some polyphenols, such as resveratrol, have shown significant activity against microsporidia (Leiro et al. 2004) and specifically against *N. ceranae* (Maistrello et al. 2008). As such, the decreasing effect on *N. ceranae* development obtained for higher concentrations of laurel and yerba mate extracts on day 7 p.i. should be studied in future experiments.

It should be noted that the substances administered may have different effects if they are fed to bee colonies. Therefore, it is necessary to test the studied substances under natural conditions, where the protein flow, survival of brood, and interaction with pollen and ventricular microbiota are variables to consider. Also, association of antibiotics and natural compounds showed synergistic activity on honeybee pathogenic bacteria (Gende 2009), therefore interactions with drugs such as oxitetracycline and fumagillin, commonly administered in field colonies, would also be interesting to study.

The significant antiparasitic activity of laurel, coupled with its palatability and low toxicity noticed during the assay, makes its inclusion feasible in a compound for nosemosis control. Ethanol extracts often have low antimicrobial action in relation to essential oils (Nanasombat and Lohasupthawee 2005; Gende et al. 2008). For this reason, it will be interesting to test compounds isolated from this herb.

ACKNOWLEDGMENTS

The authors wish to thank CONICET, UNMdP. This research was supported by ANPCyT (FONCyT), project PICT 890/06, to M.E. We thank the support provided by the 426 Animal Production Department, EEA INTA, Balcarce, the Fares Taie Laboratories Food Division and the contribution made by Enrique Tkacik.

Evaluation in vivo de l'activité anti-parasitaire d'extraits de plantes sur *Nosema ceranae* (Microsporidia)

***Apis mellifera* / *Nosema ceranae* / extrait de plante / traitement anti-parasitaire**

Eine in vivo Methode zur Beurteilung der antiparasitischen Wirkung von Pflanzenextrakten gegen *Nosema ceranae* (Microsporidia)

***Apis mellifera* / *Nosema ceranae* / Pflanzenextrakt / antiparasitische Behandlung**

REFERENCES

Adamczyk, S., Lázaro, R., Pérez-Arquillué, C., Conchello, P., Herrera, A. (2005) Evaluation of residues of essential oil components in honey after different anti-Varroa treatments. J Agric Food Chem. 53,10085–10090. doi:10.1021/jf051813f

Adler, L.S. (2000) The ecological significance of toxic nectar. Oikos **91**, 409–420

Ahameethunisa, A.R., Hopper, W. (2010) Antibacterial activity of Artemisia nilagirica leaf extracts against clinical and phytopathogenic bacteria. BMC Comp Altern Med **10**, 6. http://www.biomedcentral.com/1472-6882/10/6

Ankri, S., Mirelman, D. (1999) Antimicrobial properties of allicin from garlic. Microbes Infect **2**(2), 125–9 doi:10.1016/S1286-4579(99)80003-3

Ankri, S., Miron, T., Rabinkov, A., Wilchek, M., Mirelman, D. (1997) Allicin from garlic strongly inhibits cysteine proteinases and cytopathic effects of *Entamoeba histolytica*. Antimicrob. Agents Chemother. **10**, 2286–2288

Berbehenn, R.V., Martin, M.M. (1994) Tannin sensitivity in larvae of *Malacosoma disstria* (Lepidoptera): roles of the pertitrophic envelope and midgut oxidation. J. Chem. Ecol. **20**, 1985–2001

Block, E. (1992) The organosulfur chemistry of the genus Allium -Implication for the organic chemistry of sulfur. Angew. Chem. Int. **31**, 1135–1178

Cantwell, G.E. (1970) Standard methods for counting nosema spores. Am. Bee Jj. **110**, 220–223

Costa, C., Lodesani, M., Maistrello, L. (2010) Effect of thymol and resveratrol administered with candy or syrup on the development of *Nosema ceranae* and on the longevity of honeybees (*Apis mellifera* L.) in laboratory conditions. Apidologie **41**, 141–150

Damiani, N. (2010) Control del parásito *Varroa destructor* (Acari: Varroidae) en colmenas de *Apis mellifera* (Hymenoptera: Apidae). Doctoral thesis. School of Exact and Naturals Science. National University of Mar del Plata. Argentina (in Spanish)

Ertürk, O. (2006) Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. Sect. Cell. Mol. Biol. **61**, 275–278

Fries, I. (2010) *Nosema ceranae* in European honey bees (*Apis mellifera*). J Invertebr. Pathol. **103**, 73–79

Gende, L.B. (2009) Principales componentes de aceites esenciales relacionados con actividad antimicrobiana frente a *Paenibacillus larvae*. Doctoral thesis. School of Pharmacy and Biochemistry. University of Buenos Aires. Argentina (in Spanish).

Gende, L.B., Principal, J., Maggi, M.D., Palacios, S.M., Fritz, R., Eguaras, M.J. (2008) Extracto de *Melia azedarach* y aceites esenciales de *Cinnamomun zeylanicum*, *Mentha piperita* y *Lavandula officinalis* como control de *Paenibacillus larvae*. Zoot. Trop. **26**, 151–156

He, X.G. (2000) On-line identification of phytochemical constituents in botanical extracts by combined high-performance liquid chromatographic–diode array detection–mass spectrometric techniques. J. Chromatogr. **880**, 203–232

Higes, M., García-palencia, P., Martín-Hernández, R., Meana, A. (2007) Experimental infection of *Apis mellifera* with *Nosema ceranae* (Microsporidia). J. Invertebr. Pathol. **94**, 211–217

Keeling, P.J., Fast, N.M. (2002) Microsporidia: Biology and evolution of highly reduced intracellular parasites. Annu. Rev. Microbiol. **56**, 93–116

Lawson, L.D. (1993) Bioactive organosulfur compound of garlic and garlic products: role in reducing blood lipids. In: Kinghorn, A.D., Balandrin, M.F. (eds.) Human medicinal agents from plants, pp. 306–330. American Chemical Society, Washington

Lawson, L.D., Block, E. (1997) Comments on garlic chemistry stability of s-(2-propenyl) 2 propene-1-sulfinothioate (allicin) in blood solvents and stimulated physiological fluids. J. Agric. Food. Chem. **45**, 542

Leiro, J., Cano, E., Ubeira, F.M., Orallo, F., Sanmartìn, M.L. (2004) *In vitro* effects of resveratrol on the viability and infectivity of the Microsporidian *Encephalitozoon cuniculi*. Antimicrob. Agents Ch. **48**, 2497–2501

Maistrello, L., Lodesani, M., Costa, C., Leonardi, F., Marani, G., Caldon, M., Mutinelli, F., Granato, A. (2008) Screening of natural compounds for the control of nosema disease in honey bees (*Apis mellifera*). Apidologie **39**, 436–444

Martín-Hernández, R., Meana, A., Prieto, L., Martínez, S.A., Garrido-Bailón, E., Higes, M. (2007) Outcome of Colonization of *Apis mellifera* by *Nosema ceranae*. Appl. Environ. Microbiol. **73**, 6331–6338

Mayack, C., Naug, D. (2009) Energetic stress in the honey bee *Apis mellifera* from *Nosema ceranae* infection. J. Invertebr. Pathol. **100**(3), 185–188

Mirelman, D., Monheit, D., Varon, S. (1987) Inhibition of growth of *Entamoeba histolytica* by Allicin, the active principle of garlic extract (*Allium sativum*). J. Infect. Dis. **156**, 243–244

Nanasombat, S., Lohasupthawee, P. (2005) Antibacterial activity of crude ethanolic extracts and essential oils of spices against salmonellae and other enterobacteria. Sci. Tech. J. **5**, 462–469

Nejad, B.S., Deokule, S.S. (2009) Anti-dermatophytic activity of *Drynaria quercifolia* (L.). Jundishapur. J. Microbiol. **2**, 25–30

Ong, E.S. (2004) Extraction methods and chemical standardization of botanicals and herbal preparations. J. Chromatogr. **812**, 23–33

Paxton, R.J., Klee, J., Korpelab, S., Fries, I. (2007) *Nosema ceranae* has infected *Apis mellifera* in

Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie **38**, 558–565

Pohorecka, K. (2004a) Effect of standardized plant herb extracts on general condition of the honeybee (*Apis mellifera* l.). Bull. Vet. Inst. Pulawy. **48**, 415–419

Pohorecka, K. (2004b) Laboratory studies on the effect of standardized *Artemisia absinthium* L. extract on *Nosema apis* infection in the worker *Apis mellifera*. J. Apic. Sci. **48**, 131–136

Porrini, M.P., Audisio, M.C., Sabaté, D.C., Ibarguren, C., Medici, S.K., Sarlo, E.G., Garrido, P.M., Eguaras, M.J. (2010) Effect of bacterial metabolites on microsporidian Nosema ceranae and on its host Apis mellifera. Parasitol. Res. **107**, 381–388

Porrini, M.P., Sarlo, E.G., Medici, S.K., Garrido, P.M., Porrini, D.P., Eguaras, M.J. (2011) *Nosema ceranae* development in *Apis mellifera*: Influence of diet and infective inoculum. J. Apic. Res. **50**(1), 35–41

Rinderer, T.E. (1976) Honey bees: individual feeding of large numbers of adult workers. J. Econ. Entomol. **69**, 489–491

Rodríguez Vaquero, M.J., Tomassini Serravalle, L.R., Manca de Nadra, M.C., Strasser de Saad, A.M. (2009) Antioxidant capacity and antibacterial activity of phenolic compounds from argentinean herbs infusions. Food Control. **21**, 799–785

Soylu, E.M., Tok, F.M., Soylu, S., Kaya, A.D., Evrendilek, G.A. (2005) Antifungal activities of essential oils on post harvest disease agent *Penicillium digitatum*. Pak. J. Biol. Sci. **8**, 25–29

Tariq, K.A., Chishti, M.Z., Ahmada, F., Shawl, A.S. (2009) Anthelmintic activity of extracts of *Artemisia absinthium* against ovine nematodes. Vet. Parasitol. **160**, 83–88

Williams, G.R., Sampson, M.A., Shutler, D., Rogers, R. (2008) Does fumagillin control the recently detected invasive parasite *Nosema ceranae* in western honey bees (*Apis mellifera*)? J. Invertebr. Pathol. **99**, 342–344

Yoshida, M., Fuchigami, M., Nagao, T., Okabe, H., Matsunaga, K., Takata, J., Karube, Y., Tsuchihashi, R., Kinjo, J., Mihashi, K., Fujioka, T. (2005) Antiproliferative constituents from Umbelliferae plants VII. Active triterpenes and rosmarinic acid from Centella asiatica. Biol. Pharmacol. Bull. **28**, 173–175