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Original article

Evaluation of some essential oils for the control and prevention of American Foulbrood disease in honey bees#

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Abstract – American Foulbrood (AFB), caused by the spore-forming bacterium *Paenibacillus larvae larvae,* is the most serious disease of honeybees. Laboratory and field trials were conducted to evaluate the effectiveness of essential oils from savory, thyme, lemon-grass, and oregano, and blends of lemon-grass + thyme; lemon-grass + thyme + oregano + basil, and lemon-grass + thyme + basil in preventing and controlling infections of AFB in *Apis mellifera* colonies. The principal components of the essential oils used were determined by gas chromatography. LD_{50} values for acute oral toxicity of the oils on adult bees verified that the essences were non-toxic or slightly toxic. Results from field trials indicate that neither the essences nor the blends were effective in the elimination of AFB clinical symptoms at any dose formulation or method of administration tested, whereas tylosin was highly effective in eliminating AFB clinical symptoms.

American foulbrood / *Apis mellifera* / *Paenibacillus larvae larvae* / essential oil / tylosin

1. INTRODUCTION

American Foulbrood (AFB) caused by the spore-forming bacterium *Paenibacillus larvae larvae* is the most serious disease of bacterial origin affecting the larval and pupal stages of honey bees. In areas where disease incidence is high, antibiotics have been applied as an alternative to burning affected beehives. However the use of antibiotics risks contaminating and diminishing the quality of honey.

In vitro tests have demonstrated that some essential oils extracted from aromatic plants possess antimicrobial effect against *P. l. larvae* strains (Alippi et al., 1996; 1999b; Calderone et al., 1994; Floris et al., 1996). These compounds have also been used in colonies for the control of chalkbrood, caused

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by the fungus *Ascosphaera apis* (Colin et al., 1989) and for the control of varroasis caused by the mite *Varroa destructor* (Kraus et al., 1994; Imdorf, 1994).

The objectives of the present study were to determine the acute oral toxicity, expressed as lethal dose 50 (LD_{50}), of some pure essential oils and blends of essences on honey bees and to evaluate the response of colonies infected with AFB when treated with different formulations.

2. MATERIALS AND METHODS

2.1. Plant material and preparation of extracts

The species selected from which to extract essential oils were thyme *(Thymus vulgaris* L., Lamiaceae), lemon-grass *(Cymbopogon citratus* (D.C.) Stapf., Poaceae), oregano *(Origanum vulgare* L., Lamiaceae), basil *(Ocimun basilicum* L., Lamiaceae) and savory *(Satureia hortensis* L., Lamiaceae). Samples of savory were from Calingasta, province of San Juan, Argentina and the other species were from the area of La Plata, Province of Buenos Aires, Argentina.

Essential oils were extracted by steam distillation with a Clevenger apparatus (Guenther, 1948). In the case of thyme, savory, basil and oregano aerial parts (leaves, flowers and stems) were used; for lemon-grass, only leaves were used. The analysis of the main components was done by gas chromatography on methyl silicone column, and on a column coated with Carbowax 20 M using flame ionization detectors (FID) (Bandoni et al., 1993). The components were quantified by measuring area percentages under the peaks, and main peaks were identified by comparison with chemical standards. Formulations of essential oils were made by mixing them in sterile double distilled water and propylene glycol at 5% v/v as an emulsifier.

2.2. First acute oral toxicity test on adult honey bees: pure essences

Oral toxicity of essential oils to adult honey bees was evaluated by a technique proposed by Alippi et al. (1999a). Briefly, foraging bees were collected without using smoke and anesthetized with CO_2, placed in test flasks (10 bees per flask) and allowed to recover spontaneously. A complete randomized design, with 30 treatments and 10 repetitions, was employed. Bees remained unfed for 3 h before treatments. The 10 bees in each flask were fed 200 µL sucrose solution (50% wt/vol in distilled water) prepared with the appropriate amount of essence, so each bee consumed ad libitum about 20 µl. Concentrations of essential oils were expressed as micrograms of essential oil (e.o.) per bee (µg e.o./bee), and the treatments were: (1) savory: 5, 10, 20, 40, 80 and 160 µg e.o./bee; (2) thyme: 2, 4, 8, 16, 32 and 64 µg e.o./bee; (3) lemon-grass: 1, 2, 4, 8, 16, 32 µg e.o. /bee and (4) oregano 3, 6, 12, 24, 48, and 96 µg e.o./bee, respectively. Doses were selected according to standard bioassay procedures to estimate the LD_{50} values (Cox, 1970) according to previous in vitro tests for determination of the Minimal Inhibitory Concentrations (MIC) (Alippi et al., 1996). Dimethoate was used as a toxic standard at 0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 µg a.i. /bee (Gough et al., 1994). The non-toxic control was 50% (p/v) sucrose in water-solution. Mortality was observed after 24 and 48 hours. Lethal dose 50 (LD_{50}) (Cox, 1977) was calculated by PROBIT- and LOGIC-analysis (Collet, 1981), using Abbot's formula to correct mortality data (Abbot, 1987).

2.3. Second acute oral toxicity test on adult honey bees: essential blends

In this test, 24 treatments were given (with 10 repetitions each) using three blends of essential oils (m.e.), and dimethoate as toxic standard in 6 different doses. The methodology, statistics, and doses of dimethoate were the same as those used in the first toxicity test. Treatments were: (a) 20% lemon-grass + 80% thyme at 0.19; 0.37; 0.75; 1.50; 3.0 and 6.0 µg m.e./bee; (b) 5% lemon-grass + 11% thyme + 21% savory + 26% oregano + 37% basil at 1,19; 2.37; 4,75; 9,50; 19.0 and 28.0 µg m.e./bee and (c) 10% lemon-grass + 20% thyme + 70% basil at 0.625; 1.25; 2.5; 5.0; 10.0 and 20.0 µg m.e./bee.

2.4. First field experiment: use of essential oils as a curative treatment

A field experiment was conducted during 1995–1996 at the Faculty of Agricultural Science, UNLP, La Plata 35° S latitude, 57° W longitude. Twenty five nucleus colonies (nucs) of honey bees derived from *Apis mellifera ligustica* L. were used. Each nuc contained 18 000 adult bees, 5 combs of brood (3 combs of sealed brood and 2 combs of open brood), 2 combs with honey and pollen and 3 combs containing wax foundation. Queens were marked and their wings clipped to avoid swarming.

Colonies were distributed in a completely randomized design. The experimental procedures from inoculation to treatment, was conducted as described in Alippi et al. (1999a), with modifications. Briefly, brood comb sections (5 × 5 cm) were cut from frames from colonies with AFB clinical symptoms. Each comb section contained 45 ± 5 scales (20% of the cells of both sides) and was inserted in the central part of the central comb (experimental unit). Thirty-five days after the inoculation, disease symptoms developed on both sides of the central comb. This central comb containing various stages of brood was placed in a queen excluder, the queen remaining out of the excluder to avoid egg-laying, but allowing the entrance of nurse and house bees, this point was considered as day 0 of the experiment. This situation remained for 22 days to allow the emergence of all the brood. At the end of this period, all the colonies attached the comb section to the rest of the comb in the frame. The experimental unit was divided by wires into two areas, one containing 600 cells (small area) that included the 5 × 5 cm inoculated section, and another area of 400 cells around that, giving a total area of 1 000 cells (big area) on both sides. At the same time, the queen was confined in the central comb in the queen excluder to obtain larvae of homogeneous age and treatments were applied. Candies of 200 g (40 g of liquid honey and 160 g sugar) to which the different essential oils were added were placed on top of the frames. Five treatments with 5 repetitions per treatment were tested: Treatment 1: control; Treatment 2: 2 g savory; Treatment 3: 1 g lemon-grass; Treatment 4: 3 g oregano; Treatment 5: 1 g thyme. Doses were selected according to the results of previous in vitro tests for MIC determination (Alippi et al., 1996). After 6 days, the queen was removed and confined in another brood comb in the queen excluder in a lateral position for 8 days and the experimental unit remained free in a central position. After this period, the queen was freed to re-establish normal growth of the colony. Evaluation was made on day 48 of the experiment (15 days after the candies were placed), considering three variables for the statistic analysis: total brood area, number of larvae affected by AFB and the relation between both of them. In all cases the evaluation was carried out on the big and small areas and on both sides of the central brood comb. A linear model describing the observations was proposed to calculate ANOVA and means were compared by Tukey test.

After 12 days and every fifteen days, the residual level of the candies was evaluated, applying ANOVA repeated measures analysis and a posterior comparison of means according to LSD method (Least Square Differences) ($P > 0.05$).

2.5. Second field experiment: use of essential oils as preventive application

The second experiment was conducted from March to October 1997. The colonies contained 13 000 adult bees, 3 brood combs and 2 combs of honey and pollen. Essential oils doses were: (1) 3.6 g oregano, (2) 1.2 g thyme, (3) 1.8 g savory, (4) 1.2 g lemon-grass, (5) untreated negative control, and (6) 1.5 g tylosin tartrate used as positive control. The treatments were prepared by adding the active ingredient to 55 g powdered sugar + 15 g melted honey. Each colony was given five repetitions of the six treatments (including both controls). The first candy was given as a preventive 15 days before inoculation, which was carried out by cutting comb sections (approximately 5 × 5 cm) from frames from colonies containing AFB clinical symptoms. The introduced comb sections contained 20 ± 5 scales (10% of the cells of both sides) and were inserted in the central part of the comb.

Inoculated colonies were checked five times a month starting 30 days after inoculation. The effectiveness of the treatments was evaluated by noting the proportion of colonies with clinical symptoms of the disease (AFB-infected colonies/total of colonies) and the infection level; both variables were analyzed by separate ANOVA analysis and their means compared using a Tukey test. The AFB infection level was determined according to a scale developed by Peng (Peng et al., 1996) with the following modifications: level 0 = non detectable AFB symptoms; level 1 = 1–10 AFB-infected larvae; level 2 = 11–30 AFB-infected larvae; level 3 = 31–99 AFB-infected larvae, and level 4 = more than 100 AFB-infected larvae.

Each candy was weighed weekly to determine the amount that was consumed and was replaced with new one every 15 days. Evaluations were made every fortnight for 150 days (nine evaluations in total). The amount of candy remaining for each treatment at 7 and 14 days after application was analyzed by ANOVA repeated measures and their means were compared by LSD.

2.6. Third field experiment: preventive application of essential oil blends applied as candies

The experiment was carried out from April to August, 1999. Management, inoculation, evaluation of beehives, and statistical analysis were similar to those of the second experiment.

Four periods of inspection took place once a month after inoculation. Applications of essential oil blends, each prepared as 70 g candies, were

given every fortnight for 150 days (nine applications in total). Each candy was weighed and replaced by a new one to determine the amount of treatment remaining after 15 days. The first candy was given as a preventive 15 days before inoculation.

The following five treatments were given with 5 repetitions per treatment: Treatment A: (20% lemon-grass + 80% thyme (0.437 g of blend/every fifteen days); treatment B: 5% lemon-grass + 11% thyme + 21% savory + 26% oregano + 37% basil (3.32 g of blend), Treatment C: 10% lemon-grass + 20% thyme + 70% basil (1.75 g of blend), treatment D: untreated control. Only one repetition was given of treatment E: 0.75 g of tylosin tartrate, the positive (effectiveness) control. Candy residue data were analyzed by ANOVA repeated measures and their means compared by LSD.

2.7. Fourth field experiment: preventive application of essential oils blends applied in syrup

The experiment was carried out from May to October 2000. The methods were the same as those used in the third experiment, except that the treatments were as added to syrup and given monthly. The following treatments were given: Treatment 1: 750 mg tylosin tartrate dissolved in 750 mL of 50% sucrose solution syrup; Treatment 2 (Blend A): 20% lemon-grass + 80% thyme (3.375 g of blend); and treatment 3: untreated control (sucrose syrup at 50%). The tylosin treatment was given at the beginning of the experiment. Treatments 2 and 3 were monthly applied in five doses of 750 mL of 50% sucrose syrup. To enhance palatability, 0.5 mL raspberry essence was added to each flask of syrup. To determine consumption the remaining syrup was measured weekly.

3. RESULTS AND DISCUSSION

3.1. Principal components of essential oils

The major components of the essential oil constituents were within expected ranges and are summarized in Table I. The thyme oil used in these experiments was of the "thymol type" and also oregano clones contained a high percentage of thymol and borneol. The main constituent of lemon-grass oil was citral, in the case of basil the main components were linalool, eugenol, and limonene, and for savory, γ-terpinene, and carvacrol.

3.2. Oral acute toxicity test of pure essences on adult honey bees

The 24 h-LD_{50} and 48 h-LD_{50} for dimethoate were 0.27 μg a.i./bee and 0.17 μg a.i./bee. They were within the expected range for a highly toxic compound (Gough et al., 1994). LD_{50} could not be calculated for pure essences as negative curves for mortality values were observed. However, most of the mortalities took place at relatively low doses: 5 μg e.o./bee for savory, 8 μg e.o./bee for thyme, 2 μg e.o./bee for lemon-grass and 3 μg e.o./bee for oregano. These values would indicate that essences are among the products within the expected range for a moderately toxic product (ICCB, 1985). On the other hand, a low mortality at high doses may be caused by a lack of flavor which would delay its consumption in addition to the rapid evaporation of the essence, which would be the basis for negative curves.

3.3. Oral acute toxicity test with essential blends on adult honey bees

The 24 h-LD_{50} and 48 h-LD_{50} for dimethoate were 0.33 μg a.i./bee and 0.25 μg a.i./bee (within expected ranges, Gough et al., 1994). The 24 h-LD_{50} and 48 h-LD_{50} for Blend A (lemon-grass-thyme) were 15.94 μg m.e./bee and 18.75 μg m.e./bee, corresponding to a "slightly toxic product" (ICCB, 1985). The 24 h-LD_{50} and 48 h-LD_{50} for Blend C (lemon-grass-thyme-basil) were 122 μg m.e./bee and 356.8 μg m.e./bee corresponding to a "virtually non toxic product" (ICBB, 1985). On the other hand LD_{50} values could not be determined for Blend B due to the negative curve. In this test DL50 negative curves were observed from 1.5 to 2.5 μg m.e./bee values, depending on the kind of essence. Therefore we concluded that essence blends are palatable and they are accepted by bees up to these levels.

3.4. First field experiment: essential oils applied as a curative treatment

Results of the first field trial are summarized in Table II. Significant differences were found between the four essential oils and the control treatment in relation to the number of

Table I. Principal components of the essential oils used in this study determined by gas chromatography.

Compound	Percentage				
	Cymbopogon citratus	*Satureia hortensis*	*Thymus vulgaris*	*Ocimun basilicum*	*Origanum vulgare*
α-pinene	---	1.1	---	---	0.5
α-terpinene	---	3.6	1.8	---	---
α-terpineol	---	---	---	---	3.7
β-caryophyllene	---	1.9	1.5	---	1.5
β-pinene	---	2.2	2.0	---	1.1
γ-pinene	---	---	0.8	---	---
γ-terpinene	---	40.4	13.1	---	7.0
borneol	---	0.3	---	---	14.1
carvacrol	---	35.5	2.4	---	0.4
Citral	66.7	---	---	---	---
eugenol	---	---	---	15.8	---
limonene	---	---	---	9.4	---
linalool	---	---	2.4	42.3	1.6
myrcene	0.8	---	---	---	---
p-cymene	---	4.8	18.1	---	3.0
terpinolene	---	---	---	---	1.5
thymol	---	0.3	39.9	---	25.1
tricyclene	---	1.4	1.3	---	0.7
non identified	32.5	8.5	16.7	32.5	39.8

AFB-infected larvae and also in the relationship between AFB-infected larvae/healthy brood in the small area ($P = 0.097$). Tukey test showed that none of the essences were effective for disease control. On the contrary, these beehives showed higher levels of infection than the control beehives, the doses and the applications. Likewise, Krauss et al. (1994) demonstrated that when working with adult bees, essential oils were not effective to control *Varroa jacobsoni* in a curative way with doses between 1–3 g.

Significant differences were found ($P = 0.00$) between essences with and without control when analyzing residues of different essences after 12 days. Savory and oregano were the least consumed essences and the LSD test showed that they did not differ significantly. Thyme consumption did not differ from that of savory. Lemon-grass was the most consumed essence and it differed from the others. The bees took less than 12 days to consume the 200 g essential candies in controls, 20 days in lemon-grass, 85 days in thyme, 90 days in savory and 102 days in oregano.

On the evaluation day (15 days after consumption) colonies treated with essences showed a higher number of infected larvae than the non-treated controls. Therefore we conclude that pure essences showed a toxic effect both on the larvae and on the adult bees which made them more sensitive to disease (Lindeberg et al., 2000; Melathopoulos et al., 2000). The repellent effect of the lemon-grass on green bugs (*Schizaphis graminum*) and

Table II. First field experiment. Curative. Values of number of cells with healthy brood (eggs-larvae-pupae), number of AFB-infected larvae and relationship between AFB-infected larvae and healthy brood.

	Big area			Small area		
	AFB-diseased signs (both sides)	Healthy brood (both sides of the comb)	Relationship AFB signs/healthy larvae	AFB-diseased signs (both sides)	Healthy brood (both sides of the comb)	Relationship AFB signs/healthy larvae
F	1.57	0.96	1.36	2.39	0.91	2.54
Prob. > (F)	0.237	0.456	0.295	0.097	0.48	0.08
Mean	217.65	1575.60	0.148	138.05	1152.35	0.123
Std. Dev.	154.17	459.69	0.113	89.78	256.21	0.076
Treatments						
Savory	114.25 a	1468.75 a	0.080 a	86.25 b	1144.25 a	0.078 b
Lemon Grass	315.75 a	1290.00 a	0.218 a	211.26 b	1012.00 a	0.192 b
Oregano	241.75 a	1508.75 a	0.197 a	141.50 b	1078.00 a	0.138 b
Thyme	278.20 a	1791.20 a	0.154 a	182.80 b	1210.00 a	0.147 b
Control	91.67 a	1828.67 a	0.063 a	30.33 a	1352.33 a	0.028 a

In each column, values followed by the same letter are not significantly different at $\alpha = 0.05$.

other insects has been mentioned to depress the central nervous system (Padín et al., 2000a; Padín et al., 2000b).

3.5. Second field experiment: use of essential oils as preventive treatment

Forty five days after inoculation colonies fed with oregano showed 30% infection at level 1; with thyme 20% infection at level 2; with lemon-grass 75% infection at levels 3 and 4. No AFB signs were detected in any of the control colonies nor on the tylosin controls. Non-treated controls showed AFB infection 60 days after inoculation. The level of infection increased slightly during the test. On the other hand, in colonies treated with essences AFB began to show up in larvae 45 days -after inoculation-, with an increase in the infection level during the test. Treatment colonies fed tylosin began to show AFB infection 120 days after inoculation when two larvae were found to be affected by AFB. The colony recovered its health with no further treatment and no recurrence of the disease was observed through the end of the experiment.

The analysis of variable proportion of disease colonies (AFB-disease colonies/total number of colonies) showed significant differences due to different treatments ($P = 0.000$) but no appearent differences related to inspection periods ($P = 0.116$). The Tukey test showed that tylosin was the most effective treatment (Fig. 1A). The analysis of infection level showed that both treatments and inspection periods had significant effects (ANOVA, $P = 0.000$ and $P = 0.002$, respectively). Tylosin was the best treatment and produced significant differences on AFB-infection level (Fig. 1B); in addition, the least amount of infection was found at the first inspection period.

The analysis of the candy remnants after 7 days showed significant differences between treatments ($P = 0.001$) and between inspection periods ($P = 0.002$). Mean consumption of oregano and thyme was the lowest, with no significant differences between them. Savory, lemon-grass, control and tylosin did not differ in their mean consumption. Likewise, no significant differences were observed between treatments ($P = 0.183$) 15-days after inoculation. There were significant differences between inspection periods ($P = 0.000$).

Consumption of essence candies was improved in this second experiment, due to its dosage in six applications. Although the consumption of the treatment and the preventive applications were improved, colonies treated with essences showed higher levels of infection than with other treatments.

3.6. Third field experiment: application of essence blends in candies as a preventive treatment

Beehives treated with tylosin exhibited AFB symptoms at level 1 ninety days after inoculation. Non treated control beehives had signs of the disease 60 days after inoculation. With regard to essence blends, treatment A and C recorded 60% non infected beehives (level 0) and 40% at levels 1 and 2 after 30 days. On the other hand treatment B recorded 0% of infected colonies with level 3 and 40% at level 2. As a consequence of the disease, 16 weeks after inoculation 40% of the colonies died in all treatments except in the tylosin-treated groups.

The proportion of diseased colonies showed significant differences due to different treatments ($P = 0.000$) and also due to inspection periods ($P = 0.003$). LSD graphics showed that tylosin was the most effective treatment because it produced the smallest proportion of diseased colonies (Fig. 1C). The level of infection showed significant differences between treatments ($P = 0.000$) and inspection periods ($P = 0.003$). Again, the LSD graphics showed significant differences for tylosin treatment and for inspection period (Fig. 1D). In both analysis, the least amount of infection was found at the first inspection period.

In the comparative analysis among essence blends (without controls), results of the ANOVA showed that the proportion of diseased colonies was not related to treatments ($P = 0.244$), nor inspection periods ($P = 0.189$), therefore, no essence blend showed good performance for the control of AFB.

Significant differences were found in the residue analysis by ANOVA for treatments ($P = 0.000$) but not for inspection periods

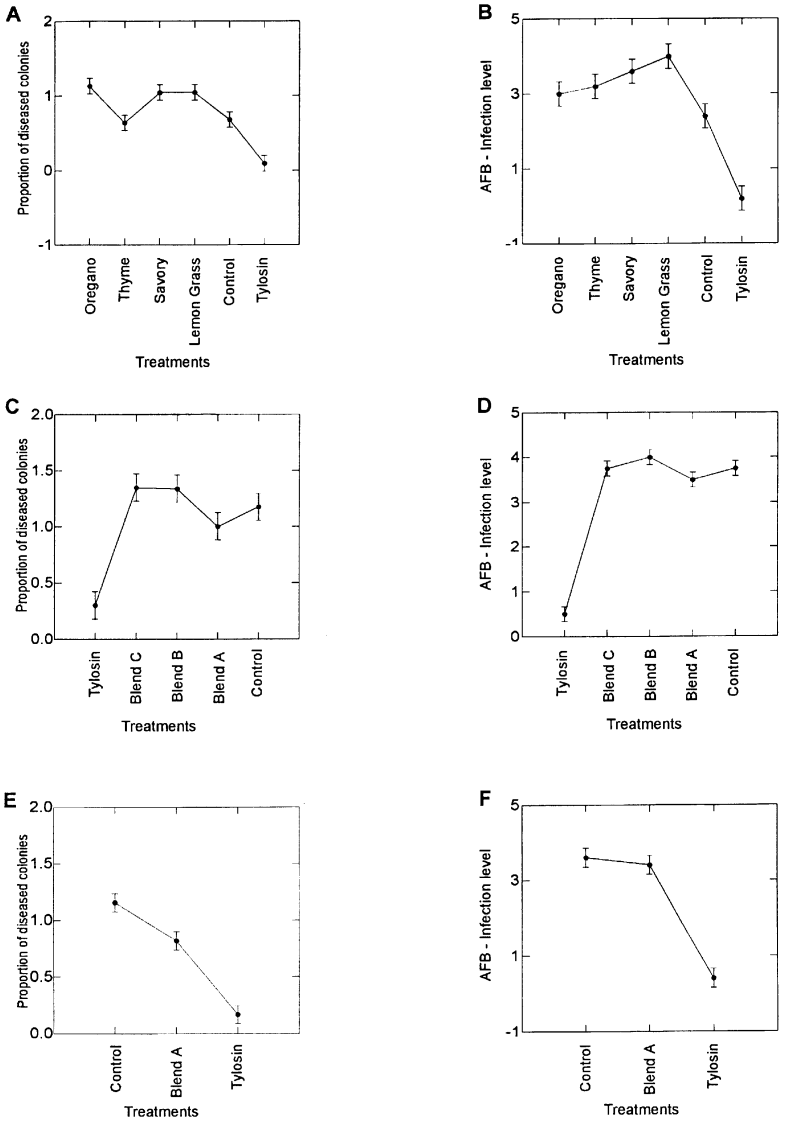


Figure 1. Field experiments. A. Second field experiment: proportion of diseased colonies infected with AFB per treatment. B. Second field experiment: AFB-infection level per treatment. C. Third field experiment. Proportion of diseased colonies infected with AFB per treatment. D. Third field experiment. AFB-infection level per treatment. E. Fourth field experiment: proportion of diseased colonies infected with AFB per treatment. F. Fourth field experiment: AFB-infection level per treatment.

($P = 0.267$). Comparison of means by LSD showed that Blend B was the least consumed with an average residue of 16.9% over 9 applications. Tylosin was excluded from the LSD analysis because the total dose was applied in an unique candy. We also observed that between the application of the first part and fourth part of the candies, the bee population decreased. This was probably due to the increased symptoms of disease throughout the time of evaluation or due to toxicity of essences to larvae and or adults.

3.7. Fourth field experiment: application of essences as syrup

Thirty days after inoculation, 33% of the treatment B colonies showed no AFB symptoms, 33% were infected at level 1, 16.5% of the colonies had infection level 2 and 16.5% had level 3 infection. Sixty six per cent of the colonies died between 60 and 120 days after inoculation. The remaining 33% had level 3 towards the end of the experiment. For treatment C, 30 days after inoculation, 33% of the colonies showed no symptoms, 50% were at level 1 and 17% were at level 3 infection. Two colonies died between 90 and 120 days after inoculation. After 90 days, treatment A (tylosin) displayed slight levels of infection, with one infected larvae (level 1), no infected larvae after 120 days (level 0) and two infected larvae (level 1) after 150 days.

The proportion of diseased colonies showed significant differences due to treatments ($P = 0.000$) but no significant differences were found related to inspection levels ($P = 0.210$). LSD graphics showed tylosin as the only effective treatment. (Fig. 1E). With regard to the level of infection, significant differences were observed due to treatments ($P = 0.000$) but not for inspection level ($P = 0.134$) Once again, LSD graphics showed tylosin as the only effective treatment for the control of AFB because it showed the lowest infection level (Fig. 1F).

Significant differences in residual syrup were found to occur at the first inspection period based on treatments ($P = 0.02$). Blend B (lemon-grass–thyme) was the least consumed.

Peng et al. (1996) observed that bees took more than 4 weeks to consume a doses of 800 mg tylosin tartrate in sugar syrup. On the other hand, 100% of 750 mg tylosin tartrate in sugar syrup was consumed in this experiment within 21 days.

Neither pure essences nor blends of essences were effective to control AFB at the doses and formulations tested in this study. On the contrary, colonies treated with essences showed higher levels of infection than those receiving control treatments. This may be related to a certain level of toxicity of the essences to larvae and adults.

The results of the present work support the effectiveness of tylosin (as tartrate) to control AFB.

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Résumé – Évaluation de quelques huiles essentielles et de leurs mélanges pour le traitement de la loque américaine. La loque américaine (AFB), causée par la bactérie sporulante *Paenibacillus larvae* subsp. *larvae*, est la maladie la plus grave du couvain de l'Abeille domestique (*Apis mellifera* L.). Dans les régions fortement touchées, au lieu de brûler le matériel infecté, on utilise des antibiotiques, mais avec le risque de contamination du miel. Il a été montré par des tests in vitro que les huiles essentielles (h.e.) extraites de plantes aromatiques possédaient une activité antimicrobienne (Alippi et al., 1999b ; Calderone et al., 1994 ; Deans et al., 1992) et certaines d'entre elles ont été utilisées avec succès pour traiter le couvain plâtré (Colin et al., 1989) et la varroose (Imdorf et al., 1994). Le but de cette étude était de déterminer la toxicité aiguë orale des h.e. pures et en mélange sur des abeilles adultes par la dose létale 50 (DL_{50}) et d'évaluer la réaction des colonies infectées par l'AFB à différents traitements basés sur les h.e. en mesurant la durée de la prise de nourriture.

Les tests ont porté sur les h.e. de (1) sarriette (*Satureia hortensis*), (2) thym (*Thymus vulgaris*), (3) citronnelle (*Cymbopogon citratus*) et (4) marjolaine (*Origanum vulgare*) et sur les mélanges suivants : (A) citronnelle + thym, (B) citronnelle + thym + origan + basilic (*Ocimum basilicum*) et (C) citronnelle + thym + basilic. Les principaux composés de chaque h.e. ont été déterminés par chromatographie en phase gazeuse sur colonne de silice méthylée et sur une colonne enduite de Carbowax 20 M et par détecteurs à ionisation de flamme (Tab. I). Pour évaluer la toxicité aiguë orale, des doses d'h.e. pures et en mélange (µg d'h.e. ou de mélange par abeille) ont été choisies conformément aux tests biologiques usuels pour déterminer les DL_{50}

(Cox, 1977). Le dimethoate a été utilisé comme référence toxique et le saccharose comme témoin non toxique.
On a fait quatre essais de plein champ, deux avec les h.e. pures, le premier à titre curatif et la second à titre préventif, et deux avec les mélanges à titre préventif. Les traitements ont été administrés sous forme de sucre candi dans les trois premiers essais et sous forme de sirop de sucre dans le dernier. On a fait cinq répétitions par traitement et utilisé deux témoins : pas de traitement (saccharose) et du tartrate de tylosine comme témoin d'efficacité, à l'exception du premier essai en plein champ qui n'a eu que le témoin non traité.
Les résultats montrent que le mélange A était légèrement toxique (DL_{50} à 24 h de 15,94 µg de mélange par abeille et DL_{50} à 48 h de 18,75 µg) et que le mélange C n'était pas toxique (DL_{50} à 24 h de 122 µg de mélange par abeille et DL_{50} à 48 h de 356,8 µg). Il n'a pas été possible de calculer les DL_{50} dans le cas du mélange B et de toutes le h.e. pures car les données de mortalité ont fourni des courbes négatives. Les résultats des essais en plein champ montrent que les h.e. pures ou en mélange ne sont pas efficaces pour éliminer les signes cliniques de l'AFB aux doses, formulations ou modes d'administration testés, tandis que le traitement à la tylosine s'est montré très efficace.

loque américaine / huile essentielle / *Paenibacillus larvae larvae* / efficacité traitement / toxicité

Zusammenfassung – Untersuchung der Eignung einiger ätherischer Öle und ihrer Mischungen zur Bekämpfung der Amerikanischen Faulbrut bei Honigbienen. Die Amerikanische Faulbrut (AFB) wird durch das sporenbildende Bakterium *Paenibacillus larvae* subsp. *larvae* verursacht und stellt die gefährlichste Krankheit der Larven- und Puppenstadien von Honigbienen (*Apis mellifera* L.) dar. In Gegenden mit starkem Befall werden als eine Alternative zum Verbrennen befallener Völker Antibiotika verwendet, hierbei besteht das Risiko einer Kontamination des Honigs. Für aus Pflanzen gewonnene ätherische Öle wurde durch in vitro – Tests eine antimikrobische Wirkung nachgewiesen (Alippi et al., 1999b; Calderone et al., 1994; Deans et al., 1992), einige wurden erfolgreich zur Bekämpfung der Kalbrut (Colin et al., 1989) und der Varroose (Imdorf et al., 1994) in Honigbienenvölkern eingesetzt. Ziel der vorliegenden Untersuchung war, die akute orale Toxizität der reinen ätherischen Pflanzenöle und ihrer Mischungen anhand der LD_{50} auf adulte Honigbienen zu ermitteln. Weiter sollte die Reaktion von mit AHB infizierten Völkern auf unterschiedliche Behandlungen mit aromatischen Ölen und ihren Mischungen unter Einschluss der Zeitdauer der Aufnahme ermittelt werden.
Ätherische Öle von (1) Minze; (2) Thymian (3) Zitronengras, und (4) Origanum und die folgenden Mischungen: (A) Zitronengras und Thymian; (B) Zitronengras, Thymian, Origanum und Basilikum, und (C) Zitronengras, Thymian und Basilikum wurden untersucht. Die Hauptkomponenten der einzelnen ätherischen Öle wurden mittels Gaschromatographie in Methylsilikonsäulen und einer mit Carbowax 20 M ummantelten Säule und Flammenionisationsdetektor bestimmt (Tab. I). Um die orale Toxizität zu bestimmen, wurden verschiedene Anwendungen der ätherischen Öle und ihre Mischungen (als µg Öl oder Ölmischung pro Biene) ausgesucht und unter Verwendung der üblichen Biotestanwendungen die LD_{50} bestimmt (Cox, 1977). Dimethionat wurde als giftiger und Rohrzucker als ungiftiger Standard verwendet.
Vier Freilandtests wurden ausgeführt, hiervon zwei mit reinen ätherischen Ölen (der erste als Heiltest, der zweite als Vorbeugungsbehandlung) und zwei mit Mischungen (beide als Vorbeugungsbehandlung). Die Behandlungen wurden in den ersten drei Tests als Futterteig, in dem letzten als Zuckersyrup geboten. Pro Behandlung wurden 5 Wiederholungen durchgeführt. Die unbehandelte Probe bestand aus Zucker, die Wirksamkeitskontrolle aus Tylosintartrat, mit Ausnahme des ersten Tests in dem nur die nichtbehandelte Kontrollgruppe geführt wurde.
Die Ergebnisse der akuten Toxizitätstests weisen darauf hin, dass die Mischung A mit 24 h-LD_{50} Werten von 15,94 µg und 48 h-LD_{50} Werten von 18,75 µg (m.e.) pro Biene m.e. leicht giftig wirkte, und dass die Mischung C mit 24 h-LD_{50} Werten von 122 µg and 48 h-LD_{50} Werten von 356,8 µg m.e. pro Biene effektiv ungiftig war. Im Fall der Mischung B und allen reinen Ölen war es nicht möglich, die LD_{50} zu ermitteln, da die Sterblichkeitsdaten negative Kurven erzeugten. Die Ergebnisse der Freilanduntersuchungen wiesen darauf hin, dass weder mit den reinen Ölen noch mit deren Mischungen und mit keiner der Formulierungen oder Dosierungen die klinischen Symptome von AFB beseitigt werden konnten, während die Behandlung mit Tylosin hocheffektiv wirkte.

Amerikanische Faulbrut / *Apis mellifera* / *Paenibacillus larvae* subsp. *larvae* / ätherische Öle

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