Efficacy and safety of Varterminator, a new formic acid medicine against the varroa mite

Matteo Giusti[a], Cristiano Sabelli[b], Alberto Di Donato[b], Devis Lamberti[b], Chiara Elisabetta Paturzo[b], Valentina Polignano[b], Roberto Lazzari[b] and Antonio Felicioli[a]*

[a]*Department of Veterinary Sciences, Pisa University, Pisa, Italy;* [b]*IZO srl, Brescia, Italy*

A new veterinary medicinal product called Varterminator for varroa mite control has been developed. The medicine is made of a gel containing formic acid (FA) and is able to produce a passive evaporation of FA and to guarantee a high degree of safety for the bee colony. To verify the efficacy of the treatment and the effect on eggs, larvae, adult bees and queens, clinical trials were performed in several apiaries spread throughout Italy, under different climatic and regional conditions. The average acaricide efficacy rate was more than 95%, with a maximum level of 99%. With regard to safety, no adverse reactions were observed on larvae, adult bees and queens when compared to untreated colonies. One adverse reaction was noted, however, a high mortality of eggs. The eggs were quickly replaced by the queen in a few days, without affecting the wellbeing of the colony. The results showed that the acaricide is efficacious and safe for colonies. The medicine can be used with brood, throughout the season of the bee activity. Moreover, the product is ready-to-use, safe for users and suitable for organic farming.

Keywords: varroa mite; formic acid; organic beekeeping; veterinary medicinal product; varroa control

Introduction

The parasitic mite *Varroa destructor* has become a widespread pest, and its control has become the most important issue in modern beekeeping (Martin, 2010; Dainat et al. 2012). Since its presumed shift from *Apis cerana* to *Apis mellifera*, mechanical, physical and chemical control approaches have been developed, following many different strategies, all leading at best to a kind of coexistence between bees and mites (Dietemann et al., 2012). Among the chemical strategies, formic acid has been used for varroa mite control since the 1980s (Ritter and Ruttner, 1980; Wachendörfer et al., 1985). Formic acid works by passive evaporation in the hive, and it is the only active ingredient able to kill the varroa mites inside the capped brood cells (Fries, 1991, Amrine and Noel, 2006). On the other hand, formic acid is considered to be widely affected by environmental temperature (Underwood and Currie, 2003), making it dangerous for the colony at high temperatures, or ineffective against varroa mites at low temperatures (Wachendörfer et al., 1985; Imdorf et al., 2003).

The aim of this research was to evaluate the acaricide efficacy and safety of a new veterinary medicinal product based on formic acid, designed to reduce the effect of environmental temperature and to guarantee an efficient and safe evaporation of the acid.

Materials and methods

Medicine

Varterminator is a new veterinary medicinal product registered in Italy. It is presented as a pad of 250 grams of 36% formic acid gel enclosed in non-woven fabric. The pads are packaged in plastic boxes which are easy to open. They must be put in the hive on the frames of colonies with normal brood conditions.

*Corresponding author. Email: antonio.felicioli@unipi.it

Table 1. Geographical coordinates, altitude above sea level, period when trial was performed and honey bee subspecies for each apiary in the study.

Place	Geographical coordinates	Altitude	Period	*A. mellifera* ssp.
Faenza, Emilia-Romagna	44°25′11′′N 11°58′36′′E	11 m a.s.l.	5 Oct-11 Nov, 2012	*A. m. ligustica*
Palermo, Sicily	37°58′25′′N 13°42′36′′E	45 m a.s.l.	25 Oct-2 Dec, 2012	*A. m. siciliana*
Ravenna, Emilia-Romagna	44°12′52′′N 11°46′06′′E	81 m a.s.l.	6 Nov-13 Dec, 2012	*A. m. ligustica*
Cagliari, Sardinia	39°21′39′′N 9°00′40′′E	46 m a.s.l.	3 Apr-10 May, 2013	*A. m. ligustica*
Massa, Tuscany	44°12′23′′N 10°04′48′′E	145 m a.s.l.	4 Jun-11 Jul, 2013	*A. m. ligustica*
Viareggio, Tuscany	43°49′45′′N 10°15′20′′E	3 m a.s.l.	8 Jul-14 Aug, 2013	*A. m. ligustica*
Belluno, Veneto	46°21′05′′N 12°10′55′′E	939 m a.s.l.	16 Jul-23 Aug, 2013	*A. m. carnica*
Belluno, Veneto	46°22′56′′N 11°58′37′′E	1,038 m a.s.l.	17 Jul-24 Aug, 2103	*A. m. carnica*
Lucca, Tuscany	43°47′44′′N 10°35′24′′E	14 m a.s.l.	6 Aug-12 Sep, 2013	*A. m. ligustica*

Trials

Field trials were performed in nine apiaries at nine sites in Italy, from October 2012 to August 2013. In each apiary, 15 colonies in Dadant Blatt hives with 10 frames were used in the trial. Six colonies were treated to evaluate the efficacy and safety of the medicine, four colonies were treated to test only the safety, and five colonies were untreated controls. In total, 90 colonies were treated and 45 were not treated. All the three subspecies of honey bee present in Italy, namely *A. m. ligustica*, *A. m. carnica*, and *A. m. siciliana* were used for the trials, according to location, but no morphological or genetic analysis was been carried out to determine the honey bee subspecies. However, for *A. m. carnica* and *A. m. siciliana* and in one case also for *A. m. ligustica* the subspecies was certified by beekeepers belonging to the official list of queen breeders for the subspecies. Each experimental group had bees of a single subspecies (Table 1).

Medicine administration

Two pads per colonies were administered for 10 days. Old pads were then removed and two new pads were administered for a further 10 days, then all pads were removed.

Varroa mite mortality and acaricide efficacy

Dead mites were checked at the diagnostic bottom of the hive every two days. To evaluate the efficacy of the new medicine, a check treatment with Apibioxal™ (active ingredient oxalic acid) under broodless conditions was performed after the end of the Varterminator administration. Broodless conditions were obtained by caging queens for 10 days from first Varterminator administration in cages model Var Control™ (5 cm × 7.8 cm × 3 cm) for 24 days. Dead mites found on the diagnostic bottom were classified as: killed by Varterminator; naturally dead; or dead by Apibioxal™, according to the day they were found.

Mites found from the 2nd day until the 32nd day after Varterminator administration were considered killed by Varterminator. Formic acid can kill foretic mites as well as mites in the capped cells (Fries, 1991; Amrine and Noel, 2006), so all mites which died during the presence of Vartermnator in the hive (from 2th to 20th days) were considered killed by formic acid. Mites which die in capped cells are not detectable until the emergence of new adult bees from the cell. Considering the developing period of a worker bee (21 days) and the ability of the queen to lay eggs only for 10 days before she was caged, dead mites detected in the 21 days after the queen caging (10th day), can be considered killed in the capped cells by formic acid. Dead mites found from 32nd to the 34th day were considered to have died naturally, because in this period they could not have been exposed to formic acid. Finally, dead mites found from the 34th day, when oxalic acid treatment was performed, to the 40th day were considered killed by Apibioxal.

The efficacy of Varterminator was calculated as [(number of mites dead by Varterminator)/(number of dead mites in whole trial)] × 100, as used in other study on formic acid efficacy (Eguaras et al., 2001). The number of the dead mite in the whole trial was calculated as: $(n_V + n_n + n_A)$ where: n_V = number of mites dead for Varterminator; n_n = number of mites naturally dead; n_A = number of mites dead for Apibioxal.

Effects on adult honey bees

The adult bee population was estimated by evaluating the area occupied by the bees on the combs, as recommended by the COLOSS *BEEBOOK*

(Delaplane et al., 2013; Dietemann et al., 2013), before the administration of the medicine and ten days after both in treated colonies and in the control colonies. Any reduction or increase in the population of treated colonies was recorded and compared with the untreated colonies.

Effects on unsealed brood

Fifty brood cells of colonies with larvae at last larval instar were marked on a transparent acetate sheet before the administration of Varterminator. After ten days, the cells were checked. Sealed cells were considered to contain live pupae, whilst in the case of empty cells, or those with a new egg, the brood was considered dead.

Effects on eggs

Fifty cells of colonies with eggs were marked on a transparent acetate sheet before the administration of Varterminator. After ten days the cells were checked. In case of the sealed cell or cell with larva at last larval instar, the egg was considered alive, in case of the empty ones, or ones replaced by a new egg, the egg was considered dead.

Effects on queens

Presence and egg-laying activity of the queen were checked before the treatment, after 10 days from administration of medicine, and at the end of the trial. Colonies without queen before the treatment were not used in the trials and were replaced by queen right ones. The age of the queens was checked by the color marker. After treatment, in case of absence of the queen, they were considered died, in case of queen present without eggs they were considered not-active.

Environmental data

Environmental data were monitored in each experimental apiary. Maximum, minimum, and average temperature and relative humidity were recorded through all the trial.

Statistical analysis

Statistical analysis for acaride efficacy, effects of the treatment on adult workers, on unsealed brood and on eggs, was performed by a 1-way ANOVA test, after the transformation of percentage values in degrees with the arcsin function, and using the least significant difference test for *post hoc* comparisons of averages. For effects on queens, the Chi-square test was used. Chi-square test was applied both on the raw data and on the same data after using the Yates' correction.

Results

Acaricide efficacy

The varroa mite mortality in the colonies treated with Varterminator was statistically higher than in the colonies of the control in all apiaries. The highest and lowest average efficacies were reported respectively in Ravenna (99.64% ± 0.04 s.e.) and in Palermo (89.05% ± 4.78 s.e.). The average efficacy of the medicine considering all the apiaries was 94.62% ± 1.23 s.e.). Efficacy of the Varterminator and natural mortality in the control in each apiary are reported in Table 2.

Effects on adult honey bee workers

After ten days from the medicine administration, both reductions and increases of adult bee populations in treated and in untreated colonies were reported. However, no significant differences were observed between treated colonies and the control (Table 3). Average value calculated on all the trial showed an increase of 1.91% ± 4.26 s.e. in colonies treated with Varterminator, and increase of 3.55% ± 2.94 s.e. in the control. This difference was not statistically significant.

Effects on unsealed brood

Unsealed brood mortality didn't show any statistical differences between colonies treated with Varterminator and control. Average mortality of unsealed brood of all trials was 9.65% ± 1.56 s.e. in treated colonies and 8.59% ± 1.58 s.e. in the control (Table 4).

Effects on eggs

In all the trials, a significantly higher mortality of eggs in the colonies treated with Varterminator compared to the control was found. Average value of egg mortality was 79.38% ± 7.31 s.e. in treated hives and 2.10% ± 0.70 s.e. in the control (Table 5).

Effects on queens

The queens involved in the study were one or two years old. No genetic relationship among queens in the same apiary was known. At the end of the trials, 11 queens had died in the 90 colonies treated with Varterminator (12.22%) compared to 5 of 45 control colonies. The Chi-square test showed no significant difference between these data. Among queens alive, only one ceased laying eggs activity after 10 days from the administration, and was replaced by the bees later on. The other live queens did not show any negative effect. Deeper investigation could be carried out to evaluate the effect of the medicine on other features of queen health as well as egg laying rhythm, oxidative stress and longevity, not observed in this work.

Table 2. Average acaricide efficacy of Varterminator and natural mite mortality in the control colonies for each apiary. Values are reported with ±standard error.

Apiary	Varterminator efficacy	Control mite mortality
Faenza, Emilia-Romagna	99.21% ± 0.28a	46.12% ± 4.97b
Palermo, Sicily	89.05% ± 4.78a	17.60% ± 3.21b
Ravenna, Emilia-Romagna	99.64% ± 0.04a	30.08% ± 1.77b
Cagliari, Sardinia	97.34% ± 0.25a	24.09% ± 3.32b
Massa, Tuscany	90.72% ± 2.28a	7.00% ± 2.92b
Viareggio, Tuscany	93.18% ± 1.78a	9.05% ± 2.13b
Belluno, Veneto	93.45% ± 0.66a	7.08% ± 0.79b
Belluno, Veneto	97.15% ± 0.24a	9.28% ± 0.50b
Lucca, Tuscany	94.58% ± 0.79a	5.20% ± 0.68b

Note: Means within the same letter are not significantly different ($p < 0.05$).

Table 3. Average reduction (−) or increase (+) of adult bee population both in treated and control colonies in each apiary ten days after Varterminator administration. Values are reported with ±standard error.

Apiary	Varterminator	Control
Faenza, Emilia-Romagna	−7.50% ± 10.08a	+1.70% ± 6.91a
Palermo, Sicily	+38.80% ± 9.02a	+27.00% ± 5.73a
Ravenna, Emilia-Romagna	−9.50% ± 4.31a	+11.00% ± 10.52a
Cagliari, Sardinia	+2.21% ± 0.79a	+1.08% ± 1.02a
Massa, Tuscany	−3.00% ± 4.39a	+7.60% ± 4.72a
Viareggio, Tuscany	+5.10% ± 1.55a	+2.70% ± 0.47a
Belluno, Veneto	0.00% ± 2.34a	−0.40% ± 0.82a
Belluno, Veneto	+0.60% ± 1.37a	+4.20% ± 2.42a
Lucca, Tuscany	+0.20% ± 2.08a	−1.00% ± 0.75a

Note: Means within the same letter are not significantly different ($p < 0.01$).

Table 4. Average mortality of unsealed brood in both treated and control colonies for each apiary. Values are reported with ±standard error.

Apiary	Varterminator	Control
Faenza, Emilia-Romagna	20.60% ± 2.17a	16.00% ± 3.91a
Palermo, Sicily	8.20% ± 1.86a	4.00% ± 3.91a
Ravenna, Emilia-Romagna	13.80% ± 1.17a	17.20% ± 4.04a
Cagliari, Sardinia	12.10% ± 1.65a	9.20% ± 1.85a
Massa, Tuscany	9.20% ± 1.53a	9.20% ± 1.85a
Viareggio, Tuscany	8.60% ± 0.85a	7.60% ± 1.32a
Belluno, Veneto	3.00% ± 1.00a	2.00% ± 1.20a
Belluno, Veneto	6.00% ± 1.63a	3.20% ± 1.20a
Lucca, Tuscany	7.00% ± 1.41a	7.60% ± 1.60a

Note: Means within the same letter are not significantly different ($p < 0.05$).

Table 5. Average egg mortality in both treated and control colonies for each apiary. Values are reported with ±standard error.

Apiary	Varterminator	Control
Faenza, Emilia-Romagna	93.40% ± 3.13a	8.00% ± 3.20b
Palermo, Sicily	63.00% ± 9.54a	3.20% ± 1.20b
Ravenna, Emilia-Romagna	94.20% ± 1.77a	7.20% ± 0.55b
Cagliari, Sardinia	94.00% ± 1.46a	7.60% ± 1.17b
Massa, Tuscany	88.40% ± 4.03a	4.80% ± 1.49b
Viareggio, Tuscany	75.60% ± 8.80a	7.60% ± 0.75b
Belluno, Veneto	69.20% ± 12.52a	3.60% ± 1.30b
Belluno, Veneto	98.40% ± 1.18a	5.60% ± 0.75b
Lucca, Tuscany	97.00% ± 1.56a	4.80% ± 2.06b

Note: Means within the same letter are not significantly different ($p < 0.05$).

Environmental data

During the administration of medicine, the highest temperature reported in all the trials was 39 °C and the lowest −1 °C. Concerning the daily average temperature, the highest was 31 °C and the lowest 9 °C. Highest value of relative humidity registered in all the trial was 89% and the lowest 43% (Table 6).

Discussion

Varterminator showed a high acaricide activity against *Varroa destructor* over a wide range of temperature without heavy negative effects for the honey bee colonies. The efficacy of this product (average value 95.64%) is similar to other registered medicines with formic acid such as BeeVar™ (Eguaras et al., 2001), Apicure™ (Hood and McCreadie, 2001), MAQS™ (Mitchell and Vanderdussen, 2010; Pietropaoli et al., 2011) or to other administration methods of formic acid (Wachendörfer et al., 1985; Feldlaufer et al., 1997; Underwood and Currie, 2005; Amrine and Noel, 2006). Varterminator showed no negative effects on adult bees, larvae or queens as has been reported for other treatments based on formic acid (Wachendörfer et al., 1985; Underwood and Currie, 2005; Amnrine e Noel, 2006; Giacomelli

Table 6. Environmental data for each apiary during each trial period.

Apiary	Temperature Max (˚C)	Min (˚C)	Average max (˚C)	Average min (˚C)	Relative Humidity R.H. max (%)	R.H. min (%)
Faenza, Emilia-Romagna	26	7	20	14	82	59
Palermo, Sicily	27	13	24	16	76	55
Ravenna, Emilia-Romagna	26	−1	20	9	89	62
Cagliari, Sardinia	27	6	19	12	82	57
Massa, Tuscany	30	13	28	18	77	47
Viareggio, Tuscany	35	17	27	23	83	56
Belluno, Veneto	38	13	29	24	71	44
Belluno, Veneto	39	14	31	24	70	43
Lucca, Tuscany	34	18	27	24	82	56

et al., 2012). However deeper investigation could be important to study potential sublethal effects on adult bees and brood.

The only negative effect noticed during treatment with Varterminator was the high mortality of the eggs. This problem could be considered not severe because the queens quickly replaced the dead eggs. The high mortality of the eggs remains an important toxic effect during the embryonic stage, but it is not as dangerous for the physiology of the colony considered as a superorganism.

Efficacy and safety of Varterminator were comparable among different localities, periods and subspecies of honey bees used in the study, suggesting that the effects of the medicine are not influenced by geographical location or honey bee taxonomy. The results showed that Varterminator is a user friendly and effective tool for varroa mite control, with high efficacy and good honey bee safety over a wide temperature range, and also suitable for organic farming systems.

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No potential conflict of interest was reported by the authors.

ORCID

Matteo Giusti http://orcid.org/0000-0002-2253-2193
Valentina Polignano http://orcid.org/0000-0001-6147-5032
Antonio Felicioli http://orcid.org/0000-0002-0808-3037

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