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SHORT COMMUNICATION

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Comparison of the efficacy of Apiguard (thymol) and Apivar (amitraz) in the control of *Varroa destructor* (Acari: Varroidae)

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Abstract

The present study compared the efficacy of Apivar (a.i. amitraz) and Apiguard (a.i. thymol) in controlling the mite *Varroa destructor* during spring 2010 and autumn 2011, in the Balearic Islands (Spain). Number of fallen mites (NFM) was counted weekly and the efficacy of treatments was evaluated by using the percentage of reduction of the average daily fallen mites (%R). During spring assay, the average NFM was highly reduced in Apiguard (89.8%) compared to Apivar (64.3%) group, with significant differences between Apiguard and control group (untreated group) in post-treatment week. In autumn assay, Apivar and Apiguard colonies had an average reduction of the NFM of 17.9% and 30.8% respectively, showing a tendency in reduction between control and Apiguard group in post-treatment week. In both assays, %R was higher in Apiguard than in Apivar, but no significant differences were found between treatments in any of the seasons. Apiguard was less efficacious during November-December, probably due to the low external temperatures that hampered an optimal volatilization of the product. The lower efficacy of Apivar is probably related to the resistance of *V. destructor* to this chemical miticide, which has been used during the last 30 years. Results of this study showed that in Mediterranean conditions, spring is an appropriate period for applying Apiguard to the colonies, whereas application in late autumn would decrease the efficacy of the product. Apiguard may represent an alternative product for integrated control due to the low risk of mite resistance and residues in bee products.

Additional key words: honeybee; *Apis mellifera*; mite control; treatment effectiveness; essential oil; falling mites.

Abbreviations used: %R (percentage of reduction of the average daily fallen mites); NFM (number of fallen mites).

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The mite *Varroa destructor* Anderson & Trueman, 2000 (Acari: Varroidae) is a hemophagous ectoparasite of honeybee (*Apis mellifera*) distributed worldwide (with the notable exception of Australia; Rosenkranz *et al.,* 2010). This parasite is currently one of the greatest menaces for apiculture (Rosenkranz *et al.,* 2010) due to economic losses and high impact on colonies health. This mite infects all the stages of honeybee, having a phoretic phase on adult bees and a reproductive phase in brood cells. Clinical symptoms are not visible at low infestation rates, and in the absence of efficacious control methods, the colonies of *A. mellifera* can collapse within a few years, in temperate climates (Boecking & Genersch, 2008). Further, *V. destructor* is responsible for the transmission of viruses to their hosts (Rosenkranz *et al.,* 2010).

In this study two chemical products commonly used for controlling *V. destructor* were compared: Apivar (a.i. amitraz, a "hard" synthetic chemical) and Apiguard (a.i. thymol, included in the group of essential oils). Amitraz was one of the earliest miticides tested against *V. destructor* (Hollingworth, 1976). Apivar possesses some advantages, such as the simplicity of application and the low economic cost. However, the main disadvantages of this product are its limited efficacy after continuous use due to the development of resistances (Trouiller, 1998; Elzen *et al.,* 1999) as well as the accumulation of residues in bee products (Martel *et al.,* 2007; Lodesani *et al.,* 2008; Chauzat *et al.,* 2009; Johnson *et al.,* 2009). Nowadays, alternative methods for *V. destructor* control based on natural products (such as organic acids and essential oils) are being

tested. Thymol (2-isopropyl-5-methylphenol) is one of those products of natural origin which have demonstrated to control varroosis both in field and in laboratory conditions (Colombo & Spreafico, 1999; Floris *et al.*, 2004; Akyol & Yeninar, 2008; Gashout & Guzman-Novoa, 2009). Apiguard acts as an efficient miticide with a low risk of residues in bee products and resistance in *V. destructor* populations (Fries *et al.*, 1991; Floris *et al.*, 2004; Bogdanov, 2006). The aim of the present study was to compare the efficacy of these two commercial products: Apiguard (thymol, essential oil) and Apivar (amitraz, formadimine), during different seasons (spring and autumn-winter) in the Mediterranean climate.

We present here the results of the study conducted in an apiary located at the University of the Balearic Islands in Majorca (39º 38' 30.20''N, 2º 38' 23.57'' E), Western Mediterranean. The apiary had 15 Langstroth standard colonies of *Apis mellifera iberica*, naturally infested with *Varroa destructor* mites. Before the trial, mite infestation level was monitored to obtain three experimental groups, as homogeneous as possible. For testing miticides, treated and untreated (control) hives were randomly selected in the apiary in order to avoid any bias due to position of the hive in the apiary. Five colonies were treated with Apivar, five with Apiguard, and other five remained untreated as control. Distance between hives was the conventional one used in the apiary (1.5-2.0 m from hive to hive).

All treatments were applied following the manufacturer's instructions. For the Apivar (Veto-Pharma SA, Villebon-sur-Yvette, France) treatment, two plastic strips (21 cm × 3.5 cm each one) were inserted in the brood chamber of each hive and left for 6 weeks. In the case of the Apiguard (Vita Europe Ltd., Basingstoke, UK) treatment, one tray (10 cm diameter) was located in the top of brood frames of each hive and left for 14 days and then it was replaced by a second tray, which was left for 2 weeks more.

The assays of 2010 and 2011 started on 8 April and 11 November, respectively. The treatments were applied on 15 April 2010 and 18 November 2011. Apiguard treatment was repeated on 29 April 2010 and 2 December 2011 and removed on 13 May 2010 and 16 December 2011, whereas Apivar treatment was not repeated and was removed on 27 May 2010 and 30 December 2011. Each colony was equipped with a modified bottom board, provided with a removable tray where a vaseline sticky paper was inserted and removed every 7 days.

Counting fallen mites is a non-destructive method and it is considered good indicator of colony infestation to be used periodically (Dietemann *et al.*, 2013). Fallen mites were counted weekly, starting one week before treatment in order to estimate the initial population of *V. destructor* in each colony. Two different parameters were studied: (i) the reduction of number of fallen mites (NFM), calculated from the difference between the number of mites obtained at the beginning and at the end of the assay; (ii) the efficacy of treatments was evaluated by using the percentage of reduction of daily fallen mites (%R), according to the following formula (Henderson & Tilton, 1955; Satta *et al.*, 2005):

$$\%R = 100\,[1 - (Bc * At/Bt * Ac)]$$

where *Bt* and *At* are the data on the average daily mite fall in treated colonies before (*Bt*) and after treatment (*At*), and *Bc* and *Ac* are the same parameters in the control group. This evaluation method is probably more precise than others using the % change in infestation, because %R incorporates variation in mite population caused by several variables, such as natural mortality, natality, immigration or emigration (Floris *et al.,* 2001).

Data were analyzed by Student's t-test (for parametric statistics). In the case of spring data, a logarithmic transformation was applied in order to improving the normality of variables (SPSS v. 20.0, SPSS Inc., Chicago, IL, USA). A level of $p \leq 0.05$ was accepted as significant, and $p \leq 0.10$ as indicative of tendency.

The results of the treated groups showed a similar pattern in both seasons (Fig. 1). We observed nnly one peak for Apivar, in the first week of product application (NFM was multiplied by 4.8 in spring and by 6.2 in autumn), showing a tendency between Apivar and control groups in the autumn assay ($t = -1.447$, $p = 0.095$). Whereas Apiguard showed two high points, one the first week (increased in 2.4 in spring and 5.7 in autumn) and other in the third week (multiplied by 2.2 in spring and by 2.9 in autumn), which coincides with the replacement of the treatment. Significant differences between Apiguard and control groups in the autumn assay were found for the first week ($t = -3.681$, $p = 0.004$) and tendency was found for the third week ($t = -1.817$, $p = 0.053$). Moreover, in the autumn assay significant differences were found between Apivar and Apiguard groups in the third week ($t = -1.594$, p 0.007).

At the end of the treatments in the post-treatment week, the average NFM during spring assay was reduced 89.8% in Apiguard treatment and 64.3% in Apivar treatment, being the difference between Apiguard and control colonies statistically significant ($t = 3.302$, $p = 0.008$), and it showed a tendency ($t = 1.492$, $p = 0.089$) in the case of Apivar and control (Fig. 1). Regarding to autumn assay, the average NFM in Apivar colonies was reduced a 17.9%, meanwhile in Apiguard-treated ones the reduction was higher, a

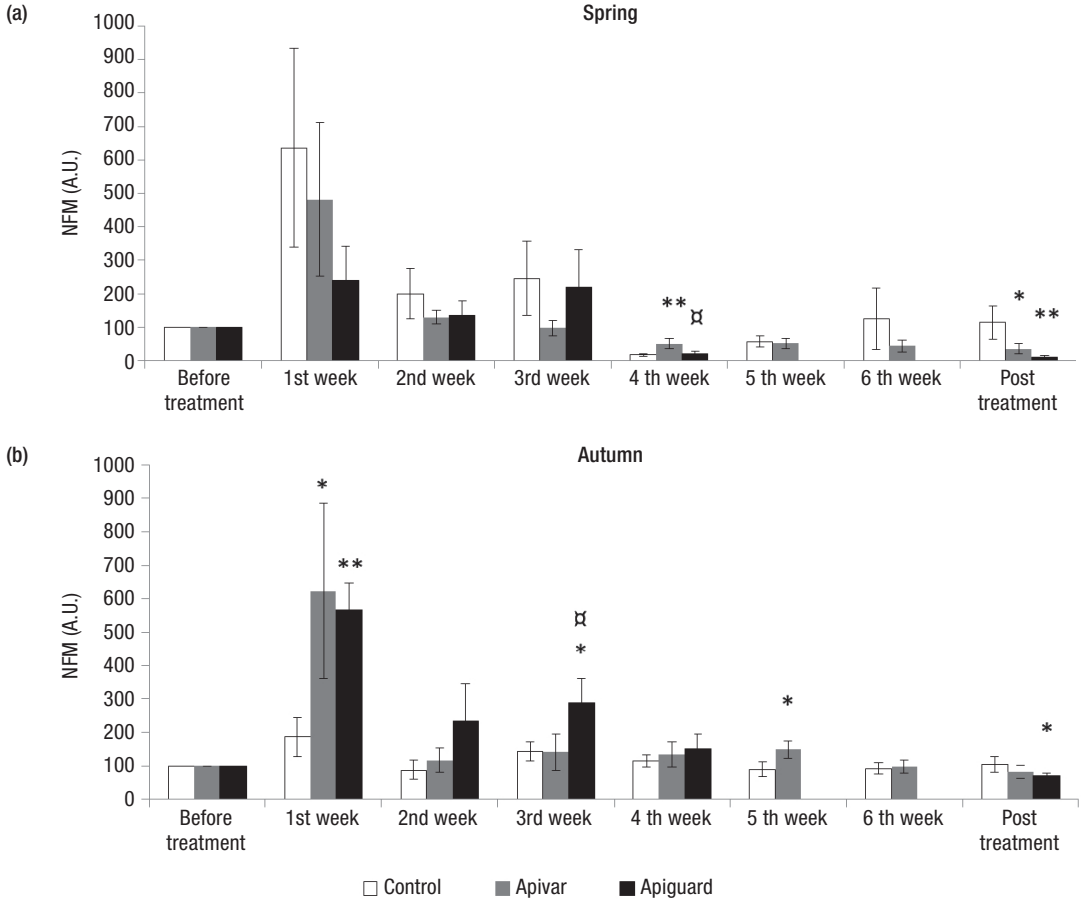

Figure 1. (a) Spring and (b) autumn assays. Data represent the means of number of fallen mites (NFM) ± SEM, before, during and after treatments. Values of weekly average NFM at t_0 (week before treatment) were set at 100, and the values of average NFM during the following weeks were represented as percentage of the NFM for week t_0. A.U.: arbitrary units. ** indicates significant differences at the $p<0.05$ level with control group. * indicates tendency at the $p<0.1$ level with control group. ¤ indicates significant differences at the $p<0.05$ level with Apivar group.

30.8%. However, in the untreated group the average NFM was increased a 5%. There were not statistically significant differences between groups, but there was a tendency (t = 1.405, p = 0.099) between control and Apiguard group (Fig. 1).

Table 1 shows the %R calculated for both treatments. Thymol-based miticide product (Apiguard) showed higher efficacy compared to amitraz-based product (Apivar). In spring the former showed a %R = 76.9%, meanwhile for the same period, Apivar showed only a %R = 65.1%, although there were not statistical significant differences between treatments (t = -0.587, p = 0.286 in spring assay and t = -0.565, p = 0.294 in autumn). Means for both treatments were ~40% lower in autumn respect to spring. The %R was also variable among colonies (Table 1).

The present work is the first conducted in the Western Mediterranean to test the efficacy of commonly used anti-varroosis treatments. Our results obtained for thymol (Apiguard) either in spring and autumn agreed with other published works where thymol showed efficacies varying from 40% to more than 90% (Marchetti & Barbattini, 1984; Chiesa, 1991; Imdorf *et al.*, 1995; Gregorc & Planinc, 2005; Loucif-ayad *et al.*, 2010). We detected a drop of efficacy of thymol in late autumn (November-December), probably due to the low external temperatures (13ºC of average daily external temperature) that hampered an optimal volatilization of the product. This observation was also reported by Ellis (2001), showing that Apiguard has a good performance at 15-20^{0}C, and lose efficacy when the temperatures are lower. In regards to amitraz, our results obtained in spring are similar to those published by Floris *et al.* (2001) and Pohorecka *et al.* (2011), who achieved an efficacy ~70%. Other authors have reported higher efficacies ranging from 82% (Chuda-Mickiewickz *et al.*, 2007; Loucif-ayad *et al.*, 2010) to 99.5% (Faucon *et al.*, 2007). One possible explanation to this lower efficacy of amitraz that needs to be tested in future works is the possibility that *V. destructor*

Table 1. Percentage of reduction of the average daily fallen mites (%R) of Apivar (amitraz) and Apiguard (thymol) treatments against *V. destructor*, during the two assays

Colony	Spring		Autumn	
	Apivar	Apiguard	Apivar	Apiguard
1	85.0	91.9	71.8	53.6
2	75.6	75.6	–36.0	27.6
3	14.4	98.0	57.1	24.7
4	51.1	24.6	14.7	10.3
5	99.3	94.6	10.9	62.6
Mean ± SEM	65.1 ± 14.9	76.9 ± 13.6	23.7 ± 19.0	35.8 ± 21.6

populations in the Balearics are resistant to amitraz due to the continuous application of this product during the last 30 years (first years with wooden frames impregnated with amitraz).

Our results also showed a high variability of treatment efficacy among colonies. This fact was already observed by other authors in Italy, Swiss and Poland (Marchetti & Barbattini 1984; Imdorf *et al.*, 1995; Colombo & Spreafico, 1999; Floris *et al.*, 2004; Chuda-Mickiewickz *et al.*, 2007). Variable efficacy among colonies could be due, in the case of thymol, to microclimatic conditions related to each colony that cause different volatilization rate and thus dispersal inside the colony; in the case of amitraz, there is emergence of resistance of *V. destructor* to this product (Trouiller, 1998; Elzen *et al.*, 1999).

Based on the results of the present study, thymol could represent an alternative in integrated control strategies against *V. destructor*, having a low risk of residues and resistance, although it may be more expensive than amitraz products (in Spain Apiguard is 86% more expensive than Apivar). Our results also pointed out that spring is the most appropriate time to apply thymol treatments to colonies managed under Mediterranean climate, whereas late autumn (November-December) seemed to be unappropriated time for an effective use of thymol. Finally, future works may include the test of other essential oils to compare with the anti-varroa effect of thymol. Further studies focused on the resistance status of *Varroa destructor* population in the Balearic Islands to widely products such as amitraz are also needed.

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References

Akyol E, Yeninar H, 2008. Controlling Varroa destructor (Acari: Varroidae) in honeybee Apis mellifera (Hymenoptera: Apidae) colonies by using Thymovar (R) and Bee-Vital (R). Ital J Anim Sci 7(2): 237-242.

Anderson D, Trueman J, 2000. Varroa jacobsoni (Acari: Varroidae) is more than one species. Exp Appl Acarol 24(3): 165-189. http://dx.doi.org/10.1023/A:1006456720416

Boecking O, Genersch E, 2008. Varroosis - The ongoing crisis in bee keeping RID C-2232-2009. J Fur Verbrauch Lebens 3(2): 221-228. http://dx.doi.org/10.1007/s00003-008-0331-y

Bogdanov S, 2006. Contaminants of bee products. Apidologie 37: 1-18. http://dx.doi.org/10.1051/apido:2005043

Chauzat M, Carpentier P, Martel A, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, Faucon J, 2009. Influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France. Environ Entomol 38(3): 514-523. http://dx.doi.org/10.1603/022.038.0302

Chiesa F, 1991. Effective control of varroatosis using powdered thymol. Apidologie 22(2): 135-145. http://dx.doi.org/10.1051/apido:19910206

Chuda-Mickiewicz B, Prabucki J, Jerzy PR, 2007. Evaluation of varroacidal efficacy of biowar preparation. J Apic Sci 51 (2): 47-53.

Colombo M, Spreafico M, 1999. Esperienze di lotta a Varroa jacobsoni Oud. con un nuovo formulato a base di timolo. La Selezione Veteterinaria 7: 473-478.

Dietemann V, Nazzi F, Martin SJ, Anderson D, Locke B, Delaplane KS, Wauquiez Q, Tannahill C, Frey E, Ziegelmann B, Rosenkranz P, Ellis JD, 2013. Standard methods for varroa research: a review. In: The coloss beebook, Volume II: Standard methods or Apis mellifera pest and pathogen research; Dietemann V, Ellis JD, Neumann P (eds). J Apic Res 52(1): 1-54. http://dx.doi.org/10.3896/ibra.1.52.1.09

Ellis JD, 2001. The future of Varroa control: Integrating current treatments with the latest advancements. Am Bee J 141(2): 127-131.

Elzen PJ, Baxter, JR, Spivak M, Wilson WT, 1999. Amitraz resistance in varroa: New discovery in North America. Am Bee J 139(5): 362.

Faucon J, Drajnudel P, Chauzat MP, Aubert M, 2007. Control of the efficacy of APIVAR ND against Varroa destructor, a parasite of Apis mellifera. Rev Med Vet-Toulouse 158(6): 283-290.

Floris I, Satta A, Garau VL, Melis M, Cabras P, Aloul N, 2001. Effectiveness, persistence, and residue of amitraz plastic strips in the apiary control of Varroa destructor. Apidologie 32(6): 577-585. http://dx.doi.org/10.1051/apido:2001145

Floris I, Satta A, Cabras P, Garau VL, Angioni A, 2004. Comparison between two thymol formulations in the control of Varroa destructor: Effectiveness, persistence, and residues. J Econ Entomol 97(2): 187-191. http://dx.doi.org/10.1093/jee/97.2.187

Fries I, Aarhus A, Hansen H, Korpela S, 1991. Comparison of diagnostic methods for detection of low infestation levels of Varroa jacobsoni in honeybee (Apis mellifera) colonies. Exp Appl Acarol 10 (3-4): 279-287. http://dx.doi.org/10.1007/BF01198656

Gashout HA, Guzman-Novoa E, 2009. Acute toxicity of essential oils and other natural compounds to the parasitic mite, Varroa destructor, and to larval and adult worker honey bees (Apis mellifera L.). J Apicult Res 48(4): 263-269.

Gregorc A, Planinc I, 2005. The control of Varroa destructor in honey bee colonies using the thymol-based acaricide - Apiguard. Am Bee J 145(8): 672-675.

Henderson C, Tilton E, 1955. Tests with acaricides against the brown wheat mite. J Econ Entomol 48(2): 157-161. http://dx.doi.org/10.1093/jee/48.2.157

Hollingworth RM, 1976. Chemistry, biological-activity, and uses of formamidine pesticides. Environ Health Persp 14: 57-69. http://dx.doi.org/10.1289/ehp.761457

Imdorf A, Bogdanov S, Kilchenmann V, Maquelin C, 1995. Apilife VAR - A new varroacide with thymol as the main ingredient. Bee World 76(2): 77-83. http://dx.doi.org/10.1080/0005772X.1995.11099245

Johnson RM, Pollock HS, Berenbaum MR, 2009. Synergistic interactions between in-colony miticides in Apis mellifera. J Econ Entomol 102(2): 474-479. http://dx.doi.org/10.1603/029.102.0202

Lodesani M, Costa C, Serra G, Colombo R, Sabatini AG, 2008. Acaricide residues in bees wax after conversion to organic beekeeping methods. Apidologie 39(3): 324-333. http://dx.doi.org/10.1051/apido:2008012

Loucif-ayad W, Aribi N, Smagghe G, Soltani N, 2010. Comparative effectiveness of some acaricides used to control Varroa destructor (Mesostigmata: Varroidae) in Algeria. Afr Entomol 18(2): 259-266. http://dx.doi.org/10.4001/003.018.0211

Marchetti S, Barbattini R, 1984. Comparative effectiveness of treatments used to control Varroa jacobsoni Oud. Apidologie 15(4): 363-377. http://dx.doi.org/10.1051/apido:19840401

Martel A, Zeggane S, Aurieres C, Drajnudel P, Faucon J, Aubert M, 2007. Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar (R) or Asuntol (R) 50. Apidologie 38(6): 534-544. http://dx.doi.org/10.1051/apido:2007038

Pohorecka K, Bober A, Skubida M, Zdanska D, 2011. Epizootic status of apiaries with massive losses of bee colonies (2008-2009). J Apic Sci 55 (1): 137-150.

Rosenkranz P, Aumeier P, Ziegelmann B, 2010. Biology and control of Varroa destructor. J Invertebr Pathol 103: S96-S119. http://dx.doi.org/10.1016/j.jip.2009.07.016

Satta A, Floris I, Eguaras M, Cabras P, Garau V, Melis M, 2005. Formic acid-based treatments for control of Varroa destructor in a Mediterranean area. J Econ Entomol 98(2): 267-273. http://dx.doi.org/10.1093/jee/98.2.267

Trouiller J, 1998. Monitoring Varroa jacobsoni resistance to pyrethroids in western Europe. Apidologie 29(6): 537-546. http://dx.doi.org/10.1051/apido:19980606