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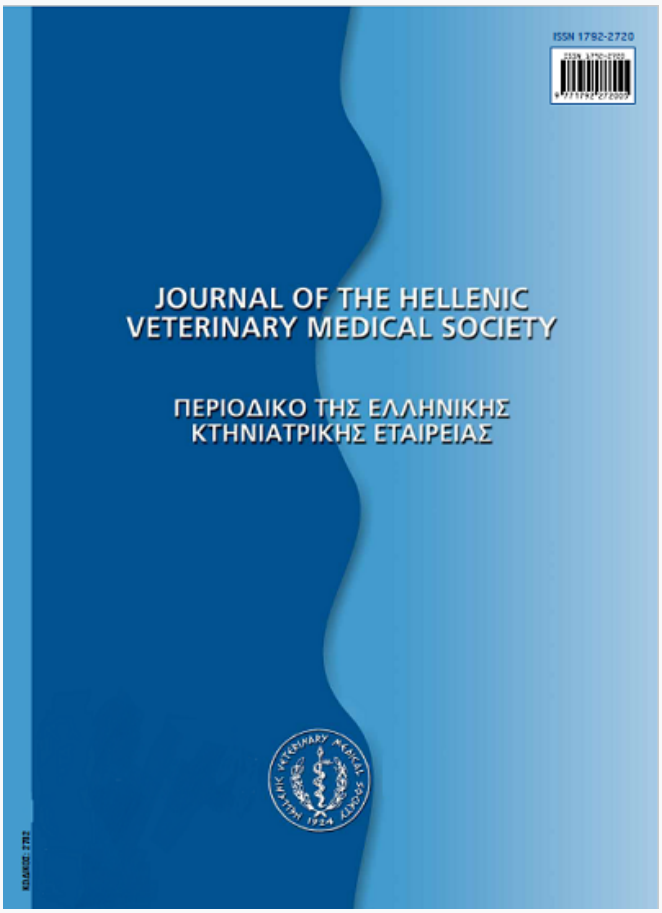

Hyperthermia -a non-chemical control strategy against varroa

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Research article
Ερευνητικό άρθρο

Hyperthermia -a non-chemical control strategy against varroa

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Υπερθερμία, μια μη χημική μέθοδος, περιορισμού της βαρροα

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ABSTRACT. Worldwide, the ectoparasitic mite varroa (*Varroa destructor* Anderson & Trueman) is potentially the main threatening parasite for *Apis mellifera* L. To find an alternative therapy for varroa and to limit the chemical residues in bee products, 27 bee colonies with their brood, were treated at 42°C for 12 to 480 minutes. All experimental colonies had 5-8 frames of brood and 10 frames of population (approximately 10.000 bees each colony). During the treatment the final temperature inside the hive

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varied from 42.3°C to 46.5°C. The effectiveness of hyperthermia to control the varroa population, depends on the duration of the therapy. When the time treatment was extended from 12 to 480 minutes, the falling mites ranged from 29.8% to 79.8%. A small number (4.7%) of dead mites was found in sealed brood after a 60-minute treatment, which gradually increased along with the treatment duration reaching 100% after 480 minutes. The use of the device irritated the bees but did not cause losses of honeybees, or excessive aggravation. Dead larvae inside sealed brood were observed when hyperthermia was applied for more than 120 minutes and increased along with the duration of heating. To our knowledge, in the presence of brood and adult bees, the hyperthermia method is used for the first time as alternative solution to limit the excessive use of chemical substances under field conditions. This method is proved to be most suitable for beekeepers with small amount of colonies.

Keywords: Hyperthermia, Alternative varroa treatment, Varroosis, *Varroa destructor*, *Apis mellifera* L.

ΠΕΡΙΛΗΨΗ Το άκαρι βαρρόα (*Varroa destructor* Anderson & Trueman) είναι παγκοσμίως η σημαντικότερη παρασιτική ασθένεια των μελισσών του είδους *Apis mellifera* L. Προκειμένου να μελετηθεί εναλλακτικός τρόπος καταπολέμησης για τη βαρρόα αλλά και να περιοριστούν τα υπολείμματα των φυτοπροστατευτικών ουσιών στα προϊόντα της μέλισσας, χρησιμοποιήθηκε η μέθοδος της υπερθερμίας. Η μέθοδος πραγματοποιήθηκε σε 27 μελίσσια παρουσία γόνου σε αυτά οπότε χρησιμοποιήθηκε η θερμοκρασία των 42°C για 12 έως 480 λεπτά προκειμένου να μελετηθεί. Όλα τα μελίσσια στα οποία εφαρμόστηκε η μέθοδος αποτελούνταν από 10 κηρήθρες πληθυσμό (κατά προσέγγιση 10.000 μέλισσες σε κάθε μελίσσι) και γόνο όλων των σταδίων σε έκταση 5 με 8 κηρήθρες. Κατά τη διάρκεια των δοκιμών η τελική θερμοκρασία στο εσωτερικό της κυψέλης κυμάνθηκε από 42,3°C έως 46,5°C. Η αποτελεσματικότητα της μεθόδου στον έλεγχο της βαρρόα, σχετίζεται με τη διάρκεια της εφαρμογής. Στο χρονικό εύρος εφαρμογής από 12 έως 480 λεπτά, το ποσοστό πτώσης ακάρεων βαρρόα κυμάνθηκε από 29,8% έως 79,8% αντίστοιχα. Ένα μικρό ποσοστό νεκρών ακάρεων (4,7%) εντοπίστηκε στον σφραγισμένο γόνο μετά από εφαρμογή 60 λεπτών, ποσοστό που σταδιακά αυξήθηκε με την αύξηση του χρόνου εφαρμογής και έφτασε έως και 100% μετά τα 480 λεπτά. Η χρήση της συσκευής εφαρμογής της υπερθερμίας προκάλεσε ανησυχία στο μελίσσι χωρίς όμως να εμφανιστούν απώλειες μελισσών ή εκτεταμένη επιθετικότητα. Εφαρμογή της μεθόδου για χρονικό διάστημα μεγαλύτερο από 120 λεπτά, προκάλεσε νέκρωση μέρους του σφραγισμένου γόνου, ενώ το φαινόμενο εντάθηκε σε έκταση σε σχέση με το χρόνο εφαρμογής. Η μέθοδος της υπερθερμίας, ως εφαρμογή απευθείας σε μελίσσι παρουσία γόνου και πληθυσμού, είναι η πρώτη φορά που μελετάται ως τρόπος καταπολέμησης του ακάρεως βαρρόα παρέχοντας έτσι μία εναλλακτική λύση στους μελισσοκόμους για τη χρησιμοποίησή της απευθείας στο μελισσοκομείο και αποφεύγοντας τη χρήση χημικών σκευασμάτων. Ως μέθοδος θα μπορούσε να εφαρμοστεί σε περιορισμένη κλίμακα και γι' αυτό προσφέρεται κυρίως για τους μελισσοκόμους εκείνους που έχουν μικρό αριθμό μελισσιών ή ασκούν βιολογική μελισσοκομία.

Λέξεις κλειδιά: Υπερθερμία, Εναλλακτική μέθοδος καταπολέμησης βαρρόα, Βαρροάτωση, *Varroa destructor*, *Apis mellifera* L.

INTRODUCTION

Varroosis has been an important cause of the decline of the bee industry around the world. The chemical detection and chemotherapy against mites have engendered inappropriate chemical residues in bee products. This has induced the search for non-chemical methods. The bee larvae are generally more tolerant to high temperatures than varroa mites. Considering this, raising the temperature inside the hive to a specific threshold would limit the varroa population while bees remain unharmed (Engels, 1994). Above the threshold temperature, the vital protein synthesis process is inhibited in the varroa mite but not in the bee larvae. Hyperthermia in a bee hive is the status of an elevated colony temperature due to failed thermoregulation. With hyperthermia only heat is used against the mite - no chemicals are involved. Additionally, to preserve the warm environment inside the colony the bees intensify abdominal pumping and wing beat activity which

causes the varroa mites to drop off (Fakhimzadeh, 2001). A large number of research findings have shown the efficacy of hyperthermia (Ruttner, 1977; Karpov, 1978; Khrust, 1978; Ritter, 1980; Solov-eva, 1983; Hoppe, 1987; Rosenkranz, 1987; Ahmad, 1988; Engels, 1992; Harbo, 1994;), but due to the lack of a suitable device or heating system it has not yet been recognized as an efficient method.

The optimum temperature for varroa development is 32°C or less (Engels, 1988) and high temperature inhibition occurs above 38°C; eventually varroa mites die at above 40°C (Rosenkranz, 1988). On the contrary, bee larvae can tolerate temperatures of 42 - 43°C (Engels, 1998) and adult bees can resist any temperature less than 48°C (Hoppe, 1986).

Even so, there are still concerns on honeybee damage from high temperature treatment; Engels (1998) stated that when the temperature in the brood nest is raised over 36°C the bees are under stress and this causes great bee losses. Precise control of the temperature and duration could result in effective varroa suppression without harming the honeybees. (Komissar, 1985; Hoppe, 1986; Rosenkranz, 1987).

For treating bee brood with heat, different types of thermostatically controlled boxes were invented, such as Borgstadter – thermo - box, Apitherm box, Varroa Controller and others. In addition, Arndt (1991) patented a device that circulates heated air at a temperature of 49°C to control Varroa (US Patent N0. 5,069,651). Glueck-Gunther (1988) also patented a device that involves blasting hot air into the hive to remove mites from bees (De Patent No. 3643872 A1). A similar device that draws air from outside, heats it and then forces it into the hive through the bottom opening is patented in Greece by Athanassakis (2006) with the name Thermovar (GR Patent No. 1005196).

In this study we tested Thermovar under field conditions. We applied hyperthermia in bee colonies without removing the brood or the population. To our knowledge this method was used for the first time. We tested the thermal treatment of 42°C at 9 different time regimes ranging from 12 to 480 min and recorded the efficiency of the method to remove mites from the bees, to kill them inside sealed brood and the side effects on bee brood and adult bees.

MATERIALS AND METHODS

Bee colony

We used bee colonies of *Apis mellifera macedonica* situated in the University farm of Aristotle University of Thessaloniki, Greece, during April-May of 2008. The environmental temperature fluctuated between 9.8°C and 17.5°C, with an average of 14.4°C in April and between 12.8°C and 25.6°C, average 19.2°C in May.

The bees were sheltered in one-storey hives, with Langstroth standard size combs where they had 10 frames population and 5-8 frames of brood. Beehives had removable screened bottom board with a drawer that allowed the observation and count of the mites dropping off the bees after each treatment. In each beehive two digital thermometers were placed in the central frames of brood to record the temperature from the beginning of the treatment to its completion.

Heating device

Thermovar is a device of adjustable temperature and time and consists of three parts: the heat chamber, the hot air shaft and the mite-collecting drawer. The heat chamber is placed on top of the beehive, after previous removal of the hive's lid and placement of a bee-safe screen (figure 1). Through the hot air shaft, the chamber is connected to the mite-collecting drawer, which is placed below the screen of the bottom board. This whole system-beehive, heat chamber, hot air shaft and mite-collecting drawer- is a closed air-circuit, except of the minimum and controlled amount of air coming in through the entry hole on the drawer and out through the exit hole on the shaft. When the device is turned on, the air inside the system begins to heat gradually and with the help of the circulator is recycled through the heat chamber – shaft – drawer – spaces between the combs of the beehive and heat chamber again. The function of this whole system is controlled by electronic instruments, thus, when the desired air temperature is reached, it remains stable for the time set.

The device temperature was adjusted to 42°C and the thermal treatment was applied on 27 complete bee colonies for a time range of 12 to 480 minutes. The duration we used in particular was 12, 20, 30, 40, 60,

120, 240, 360 and 480 minutes as shown in tables 1,2,3 according to the manufacturer observations and as objective of the experiment. The time count began when the temperature of the hive reached 42°C. For every temperature and time three different colonies were used.

Counting of varroa mites and dead bees

After each treatment the varroa in the bottom drawer was counted as well as its mortality inside the brood cells. The count of mites that had dropped off the bees, continued for 6 days after each treatment. On the seventh day the bees were treated with an effective acaricide and the dead varroa was counted once more. The efficacy of the hyperthermia was estimated as a percentage of the number of dropped mites in 6 days, on the total number of varroa in the beehive.

To find whether varroa mites that fell in the bottom drawer after the treatment were dead, irreversibly hurt or alive they were placed in a petri dish inside an incubator, set on 35°C along with bee larvae' and they were checked for the next two days.

In order to estimate the mortality of the mites inside the cells, 300 brood cells from three different combs of each colony were uncapped and the state of the mites in the cells was recorded (dead or alive).

The effect of hyperthermia on the bees was checked by counting dead bees in front of the hive and also by marking 100 brood cells from each colony and following their development. Moreover the bees' behavior (aggressiveness), queen replacement and the overall development of the bee colonies treated with hyperthermia were recorded.

RESULTS

Treated temperatures with hyperthermia

Temperature fluctuation in treated colonies and the time of treatment are summarized in Table 1. The temperature of the device was adjusted to the 42°C. The normal hive temperature which ranged between 32.2 and 36.6°C was recorded as the initial temperature. During treatment the temperature varied from 42.3 to 46.5°C. In most cases the device successfully produced the heat to reach the expected 42 to 44°C inside the hives. In 12 out of 27 cases, the final temperature exceeded the 44°C that larvae tolerated, by 0.2°C to 2.5°C. The maximum temperature in most cases was more than 50°C. This

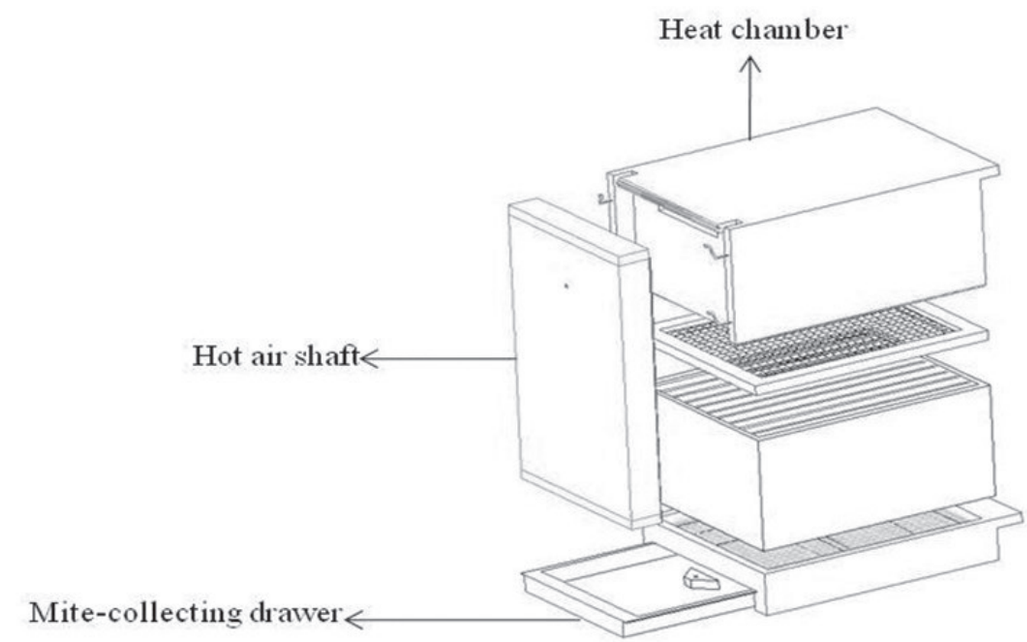

Figure 1.

Table 1. Changes of temperatures in 27 bee colonies treated with hyperthermia at 42oC

Colony	Time of treatment (min)*	Temperature inside the bee hive (°C)			Time needed to reach the temperature of 42°C
		Initial	Final	Maximum	
1.	12	33.2	43.4	50.3	16
2.	12	36.4	45.6	52.2	20
3.	12	36.2	43.3	51.8	21
4.	20	35.7	42.6	47.8	18
5.	20	36.5	42.3	50.4	22
6.	20	36.6	43.6	47.8	23
7.	30	33.3	43.9	50.4	20
8.	30	34.3	45.0	52.3	30
9.	30	33.6	43.1	50.9	19
10.	40	33.3	43.1	50.4	24
11.	40	32.2	44.5	50.0	32
12.	40	33.1	46.5	52.3	27
13.	60	35.2	44.3	52.3	15
14.	60	34.4	42.6	51.7	16
15.	60	34.3	43.5	51.6	20
16.	120	32.8	45.6	52.3	23
17.	120	33.1	44.2	50.9	21
18.	120	35.1	43.9	53.4	16
19.	240	32.3	42.5	50.8	18
20.	240	34.5	44.9	50.3	21
21.	240	32.2	45.0	50.4	30
22.	360	33.6	44.5	52.3	27
23.	360	34.6	43.2	52.8	26
24.	360	33.2	43.9	50.2	21
25.	480	34.3	43.6	49.8	18
26.	480	34.9	45.2	52.8	20
27.	480	34.2	44.8	51.3	23

**The time measured from the time that temperature inside the hive reach 42°C*

high temperature did not last long, since the bees managed to reduce it by actively evaporating water, exchanging liquid food and oxidizing carbohydrates from the honey-crop (Komissar, 1985). The time needed for the final temperature to be stabilized ranged between 15 and 32 minutes, depending on the population of the colony and the number of combs.

Mortality of dropped varroa mites onto the bottom board

Table 2 indicates the number of varroa that were dead or living 24 h after they had dropped off the bees. During shorter treatments (less than 40 min) most of the fallen mites survived. The mortality of the mites was similar for 60 to 360 minute treatments (average 26.9%) and increased to 45.5% at 480 min. Along with the increasing time of the application, the number of varroa mites falling onto the drawer of the bottom board, increased respectively (Fig. 2). The mortality of the dropped mites and the duration of the treatment were linearly related (R^2=0.86). The efficacy of the hyperthermia treatment was estimated as a percentage of the number of fallen mites in 6 days, on the total number of varroa in the beehive. Thermal treatment is relevant to the duration of the application since the enforcement for 12 to 480 minutes caused 35.9% to 79.8% dropped mites respectively.

Mortality of varroa mites in brood cells

Varroa mortality in brood cells is presented in Table 3. The total number of mites that were found in 900 cells from every experimental group (300 cells/ hive) and their mortality after the thermal treatment in different times were observed. A percentage of 12.9% of varroa mites were found dead inside brood cells after 120 minutes treatment. This percentage reached 57.8% after 240 minutes treatment, 92.6% after 360 min and 100% after 480 minutes.

By the end of the treatment and approximately for the next half an hour, an irritation was observed among the bees, though they did not have a tendency to sting, while part of the population flew out of the hive. No losses of adult bees were observed even after 480 minutes of treatment. Up to 120 minutes of treatment the brood was not affected negatively, whereas after 240 minutes, a percentage from 6% to 50% of the larvae seized to develop and died.

The development of the treated bee colonies was normal and no delay was observed in the following months compared to other colonies of the same apiary which were not subjected to hyperthermia.

DISCUSSION

We applied hyperthermia in bee colonies with the presence of brood and the adult bees, with hot air at the temperature of 42˚C. Despite the presence of bees, the temperatures inside the hive kept fairly stable between 42.3°C and 46.5°C. These temperatures are increasingly lethal to varroa mites depending on the duration of the treatment. More than 50% of the mites dropped off adult bees after 60 minute-treatment and about 76% after 480 minute-treatment (Fig.2). Kommissar (1985) applied hyperthermia successfully using an incubator (thermocamera) at 47˚C for 12-15 min on caged bees and removed more than 95% of the mites. Harbo (2000) kept honeybees for 48 hours in temperatures of 25, 35, 38, and 40˚C and found that the lowest temperature at which mites could be removed was 40˚C. Hoppe (1986) also tested 48˚C for 20 minutes in artificial swarms and removed 23% of the mites. In this research the temperature, the time of treatment, the presence of frames with honey and brood, the humidity of the hive and other factors may affect the results. Air flow should also be considered, since the apparatus we used forced hot air continuously into the hive through the bottom opening. This could explain the different results we have from Harbo (2000) who unsuccessfully treated caged bees with comb at 40˚C for 48 hours.

Previous researchers applied hyperthermia on sealed bee brood for a longer time than the one that was

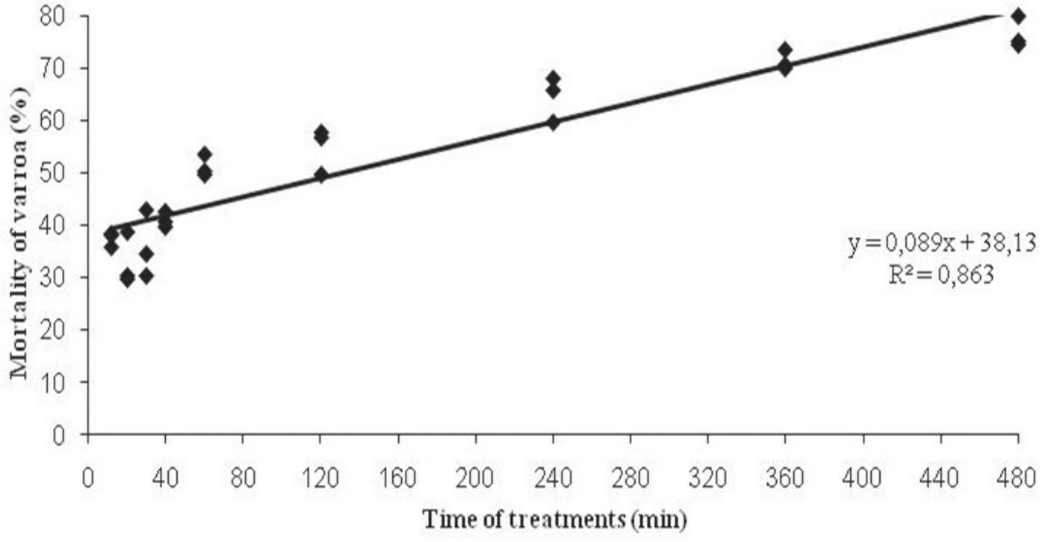

Figure 2.

Table 2. Mortality of varroa mites that dropped into the bottom board of the hives after treated with hyperthermia (total of each group)

Time of treatment (min)	Varroa found in bottom board	Dead varroa after 24 hours
12	150	0
20	114	0
30	132	6 (4.6%)
40	168	12 (7.2%)
60	102	27 (26.5%)
120	75	24(32.0%)
240	219	57 (26.0%)
360	195	45 (23.1%)
480	132	60 (45.5%)

Table 3. Mortality of varroa mites in brood cells after treatment with hot air at 42°C

Time of treatment (min)	Number of Varroa in 900 brood cells*	Number of dead Varroa in brood cells
12	84	0
20	96	0
30	24	0
40	195	0
60	129	6 (4.7%)
120	93	12 (12.9%)
240	135	78 (57.8%)
360	201	186 (92.6%)
480	159	159 (100%)

** 300 brood cells were opened from each of the three colonies that had been used in the experiment.*

used in swarms of adult bees (Rosenkranz, 1987, Engels, 1998). In this study we found few dead mites after 60 to 120 minutes of treatment and all mites were dead after 480 minutes of treatment. Rosenkranz (1987) also noted that after 45˚C treatment for 240 minutes, 80-100% of the mites were killed. The question in concern is why the fallen mites from the adult bees were not killed by 480 minute treatment and 54.5% of them survived. According to Kommissar (1985) mites may stay alive at 41°C by sucking the bees' haemolymph. Another explanation could be the effort of bees to lower the temperature of their hive by evaporating water during treatment, which may save some mites.

The ability of the survivor mites to re-enter brood cells and reproduce after hyperthermia treatment was not examined and neither was the effect of hyperthermia on their longevity. According to LeConte, et al., (1990) mites in brood cells failed to reproduce or were killed when they were exposed to 42˚C for 360 minutes.

The fact that the majority of the fallen mites do not die but they have the chance to re-infest the bees may carry the risk of the development of heat resistant varroa. According to Chacon-Almeida, et al., (2000) special proteins, the heat shock proteins, are rapidly synthesized when organisms are exposed to elevated temperatures. This must be considered carefully in practice so that the treated hives should be provided with screened bottom board or covered with a thin layer of vaseline or any other non-toxic adhesive substance.

CONCLUDING REMARKS

The treatment of bees with Thermovar can be lethal to varroa mites when applied at 42°C, depending

on the duration of the treatment. It can effectively remove great proportion (more than 90%) of the phoretic mites after 360 to 480 minutes. During treatment, bees should be confined in their hives so treating during nighttime could be the best choice.

Hyperthermia is another means to control varroa and although it had been proposed 43 years ago (Kiroazy, 1973), it lacks an established and acknowledged method of application. Application of hyperthermia can be an alternative to the use of acaricide chemicals that leave residues or volatile compounds that may disorganize bee communication. It is a rather promising method for beekeepers with a small number of colonies, regardless of the type of beekeeping they choose to practice, conventional or organic.

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