Controlling *Varroa destructor (Acari: Varroidae)* in honeybee *Apis mellifera (Hymenoptera: Apidae)* colonies by using Thymovar® and BeeVital®

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ABSTRACT

This study was carried out to determine the effects of Thymovar® and BeeVital® on reducing *Varroa* mite (*Varroa destructor*) damage in honey bee (*Apis mellifera* L.) colonies in spring season. Average percentage of *Varroa* infestation level was determined as 24.27 on adult workers before the treatments. The drugs were applied two times on 25 September and 16 October 2006. Average percentage of *Varroa* infestation levels were determined as 5.18%, 10.78% and 35.45% after the first application, 1.90%, 7.05% and 61.15% after the second application in Thymovar®, BeeVital® and control groups, respectively. Average efficacies of Thymovar® and BeeVital® were found to be 96.91% and 88.66%, respectively. Difference between drug efficacies on *Varroa* mite was found significant ($P<0.01$). There was no queen, brood and adult honeybee mortality in all group colonies during the research.

Key words: Honeybee, *Apis mellifera*, *Varroa destructor*, Thymovar®, BeeVital®.

RIASSUNTO

CONTROLLO DI *VARROA DESTRUCTOR (ACARI: VARROIDAE)* IN COLONIE DI API MELLIFERE *(HYMENOPTERA: APIDAE)* ATTRAVERSO L'USO DI THYMOVAR® E BEEVITAL®

Questo studio è stato eseguito al fine di determinare gli effetti di Thymovar® e BeeVital® sulla riduzone dei danni provocati da acari Varroa (Varroa destructor) in colonie di api mellifere (Apis mellifera L.) nella stagione primaverile. Prima del trattamento è stata determinata la percentuale media di infestazione delle api operaie adulte (24,27). Le preparazioni farmaceutiche sono state somministrate due volte, rispettivamente il 25 Settembre e il 16 Ottobre 2006. La percentuale media di infestazione da Varroa è stata: 5,18%, 10,78% e 35,45% dopo il primo trattamento e 1,90%, 7,05% and 61,15% dopo il secondo trattamento rispettivamente nei gruppi Thymovar®, BeeVital® e nel gruppo di Controllo. L'efficacia terapeutica media di Thymovar® e BeeVital® è stata 96,91% e 88,66% rispettivamente. La differenza di efficacia terapeutica su acari Varroa è stata significativa tra i due farmaci (P<0,01). Non è stata rilevata mortalità di regine, adulti o covate, nei diversi gruppi, durante la prova sperimentale.

Parole chiave: *Api mellifere,* Apis mellifera*,* Varroa destructor*, Thymovar®, BeeVital®.*

Introduction

Besides genetic structure and environmental factors, the pests, diseases and parasites of the honeybees *(Apis mellifera)* are the most important factors that influence the productivity of the honeybee colonies (Kaftanoğlu *et al.*, 1995; Fakkimzadeh, 2001). *Varroa* is the most important parasite of A. mellifera that influences the colony development and performance (Genç, 1994; Baggio *et al.*, 2004; Akyol and Korkmaz, 2005; Kar *et al.*, 2006). It is also known as the most serious problem in beekeeping all over the world (Fakkimzadeh, 2001; Goodwin and Eaton, 2001; Baggio *et al.*, 2004).

Because of the damages caused by *Varroa*, beekeepers lose a great number of colonies in winter or start with an unhealthy, weak colony in the spring season (Genç, 1994; Kaftanoğlu *et al.*, 1995; Imdorf and Carriere, 1996; Imdorf *et al.*, 2003; Akyol and Özkök, 2005). Beekeeping would be neither profitable nor enjoyable in many areas without effective treatment against *Varroa* (Çakmak *et al.*, 2003). Many chemicals have been used to reduce or eliminate the damages of the *Varroa* throughout the world (Genç and Aksoy, 1992; Milani, 1993; Goodwin and Eaton, 2001; Baggio *et al.*, 2004; Akyol and Korkmaz, 2006). Residue problems were started in honeybee products due to heavy uses of chemicals (Faucon and Flamini, 1990; Slabeezki and Lensky, 1991; Imdorf *et al.*, 2003; Donders and Cornelissen, 2005). Due to the severity of the residue problems international and national food regulations were established for he consumption and trade of honeybee products.

Efficacy of most of the chemicals decreased, because of the development of resistant mites against to the chemicals (Colin, 1990; Gerson *et al.*, 1991; Milani, 1995; Fakkimzadeh, 2001; Imdorf *et al.*, 2003). Because of the residue problems research on using of natural products, such as organic acids and components of essential oils (e.g. thymol), has been intensified for the control of *Varroa* mite (Robaux, 1986; Colin, 1990; Calderone *et al.*, 1991; Chiesa, 1991; Rickli *et al.*, 1991; Imdorf and Carriere, 1996; Bogdanov *et al.*, 1999; Eugaros *et al.*, 2001; Fakkimzadeh, 2001; Baggio *et al.*, 2004). *Varroa*cidal activity of thymol was shown in both laboratory assays and in field studies in Europe and in North America (Imdorf *et al.*, 1999; Mattila and Otis, 1999, 2000; Whittington *et al.*, 2000; Ellis *et al.*, 2001; Melathopoulos and Gates, 2003). Thymovar has been recommended for controlling *Varroa* in honey bee colonies (Chiesa, 1991; Rickli *et al.*, 1991; Imdorf and Carriere, 1996; Bogdanov *et al.*, 1999; Fakkimzadeh, 2001; Goodwin and Eaton, 2001). It was reported that none of the thymol treatment affected the number of adult bees and amount of brood in the hives (Imdorf *et al.*, 1995; Imdorf and Carriere, 1996). However, it is not recommended to use products with thymol ingredients during the honey flow period because it may leave taste residue in honey and the efficacy on *Varroa* mites is lower (Gal *et al.*, 1992; Imdorf *et al.*, 1995, 2003; Kaftanoğlu *et al.*, 1995; Donders and Cornelissen, 2005). It was also recommended not to use powdered thymol especially on weak colonies at high ambient temperatures (Mikityuk and Grobov, 1979). Many factors may influence the action of thymol such as environmental factors, colony condition, time of intense brood rearing and application method. In order to achieve the best effect of both Thymovar and Bee-Vital treatment, the temperature of day-time should be around 15-20°C and should not fall below 12°C for a long time (several days) and the hives should also have a small brood or no brood at all (Gal *et al.*, 1992; Imdorf *et al.*, 1995).

The aim of this study was to determine the efficacies of Thymovar® (thymol) and BeeVital®(HiveClean) on *Varroa destructor*

in honey bee (*Apis mellifera*) colonies during the autumn season in Turkey.

Material and methods

This study was carried out on 30 honeybee colonies (*Apis mellifera*) in Central Anatolia (*37°29'20" N longitude, 34°37'42" E latitude and 1260 m altitude*) between 25 September and 06 November 2006. Genetic stock, queen age, colony strength, brood areas and food stock of the colonies were equalized and all colonies were housed in the standard Langstroth wooden hives before the experiment. All colonies had an average of 7 frames of adult bees and very low brood area (average 150 cm^2/colony). The colonies were randomly divided into three groups consisting of 10 colonies per treatment group. The first group was treated with Thymovar® (15 g or 68.81% extracted thyme plant and 6.8 g or 31.19% viscose sponge), the second group was treated with BeeVital® (oxalic acid, citric acid, core of propolis, etheric oils, sucrose and water mixture) and the third group was used as the control. The percentage *Varroa* infestation levels of colonies were determined at the beginning and at the end of each drug application (Gal *et al.*, 1992; Genç and Aksoy, 1992; Goodwin and Eaton, 2001; Kumova, 2001) by using wash and roll technique (De Jong *et al.*, 1982). The *Varroa* infestation level of sealed brood area was not determined because there was not enough sealed brood area in all colonies either before or after the experiment. A high relationship was reported with respect to the *Varroa* infestation level between adult worker bees and in sealed brood (Branco *et al.*, 2006). At the beginning of the experiment one half of Thymovar® wafer was placed directly over the brood combs in the first group and 15 ml BeeVital® was trickled on adult worker bees between the frames for each colony in the second group as described in their prospectus. After three weeks the Thymovar® wafer (1/2 wafer) was replaced with a fresh one (Bolhalder, 1999) and 15 ml BeeVital® was trickled again. Daily temperature fluctuations were recorded during the experiment.

Henderson-Tilton's formula was used for determining the percentage of efficacy of the chemicals (Henderson and Tilton, 1955). The *Varroa* infestation level (%) was analysed by randomised plot design (ANOVA). Levene Statistic was used for testing variance homogeneity among dependent variables. Logarithmic transformation was done to stabilize the variances among the numbers of *Varroa* in groups after the chemical applications. Antilog transformation was performed for group means and the 95% confidence intervals of the means were calculated. Group comparisons among the means were done with Duncan's multiple range test and different statistical groups were shown in different letters in tables (Little and Hills, 1975). SPSS, 15.0 Ver. software was used for the statistical analysis.

Results and discussion

Varroa infestation levels before and after the first and second drug applications were summarized in Table 1, Table 2 and Table 3.

The average *Varroa* infestation rate (%) of all experimental colonies was found to be 24.27% at the beginning of the experiment. There were no significant differences ($P > 0.05$) among the group means in terms of *Varroa* contamination level.

Significant differences ($P<0.01$) were observed among the groups in terms of *Varroa* contamination at the end of the first application. The average number of *Varroa* on adult bees were found to be 1.90%, 7.05% and 61.15% in Thymovar®, BeeVital® and control group colonies after the second treatment. The final efficacy of Thymovar® and BeeVital® drugs were calculated as 96.91% and 88.66%, respectively.

Table 1. Average *Varroa* infestation level (%) before application in different groups.

Drugs	N.	Mean ± SE	Range	
Thymovar®	10	24.94 ± 1.57	15.79	30.95
BeeVital®	10	24.25 ± 1.77	16.24	32.84
Control	10	23.56 ± 1.95	14.93	33.66

Table 2. *Varroa* infestation levels (%) after first application in different groups.

Drugs	N.	Mean ± SE	Range	
Thymovar®	10	5.18 ± 0.41[a]	3.23	6.91
BeeVital®	10	10.78 ± 0.76[b]	7.07	14.12
Control	10	35.45 ± 2.18[c]	25.11	43.65

**Different letters indicate significant differences among the means (P<0.01).*

Table 3. *Varroa* infestation levels (%) after second application in different groups.

Drugs	N.	Mean ± SE	Range	
Thymovar®	10	1.90 ± 0.15[a]	1.41	2.75
BeeVital®	10	7.05 ± 0.41[b]	4.78	9.33
Control	10	61.15 ± 2.01[c]	51.28	74.13

**Different letters indicate significant differences among the means(P<0.01).*

The results obtained in the Thymovar® group of the current experiment are in accordance with the findings (90 - 99%) of Marchetti and Barbattini (1984)., Colin (1990), Chiesa, (1991), Rickli *et al.*, (1991), Liebig (1993), Schulz (1993) Higes *et al.* (1996), Colombo and Spreafico (1999), Bogdanov *et al.* (1998), Bollhalder (1999), Imdorf *et al.* (1994, 1995, 1999, 2003) and Baggio *et al.*, (2004). But they were found higher than those reported (68.7 – 84.8%) by Gal *et al.* (1992) and Mutinelli *et al.*, (1993), Mattila and Otis (1999, 2000), Goodwin *et al.*, (2003).

Horn (2003) tested the efficacy of BeeVital® and reported that it was 93.9% effective against to *Varroa* mite. The efficacy of BeeVital® (HiveClean) in our study (88.66%) was lower than the findings of Horn (2003).

Conclusions

Thymovar® had higher efficacy than BeeVital® (HiveClean) and it was possible to keep low *Varroa* prevalence indices in the colonies with only two applications of one Thymovar® wafer. While *Varroa* contamination of the control group increased to 61.15%, the infestation level of *Varroa* in Thymovar® group decreased to 1.90% at the end of the study. Thymovar®, may be a good alternative for *Varroa* control and it has many advantages: it is easy to use, safe for beekeepers and presents low variability between colonies in its final efficacy. No case

of honeybee toxicity, loss of queens, brood or adult honeybee mortality has been recorded. This study showed that Thymovar® and BeeVital® (HiveClean) could be used effectively and safely to reduce the damages of the mites in the honeybee colonies.

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