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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 DESTROYER (ACARI: VARROIDAE) IN COLONIE DI API MELLIFERE (HYMENOPTERA: APIDAE) ATTRAVERSO L’USO DI THYMOMAR® E BEEVITAL®

Questo studio è stato eseguito al fine di determinare gli effetti di Thymovar® e BeeVital® sulla riduzione 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% e 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; Slabezki 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 the consumption and trade of honeybee products.

Efficacy of most of the chemicals decreased, because of the development of resistant mites against 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). Varroacidal 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, 2003; Kaftanoğlu et al., 1995; Donders and Cornelissen, 2005). It was also recommended not 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 cm²/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 groups 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.
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 Sprefico (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

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### 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 |

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

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

| Drugs      | N. | Mean ± SE | Range |
|------------|----|-----------|-------|
| Thymovar®  | 10 | 5.18 ± 0.41<sup>a</sup> | 3.23 6.91 |
| BeeVital®  | 10 | 10.78 ± 0.76<sup>b</sup> | 7.07 14.12 |
| Control    | 10 | 35.45 ± 2.18<sup>c</sup> | 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<sup>a</sup> | 1.41 2.75 |
| BeeVital®  | 10 | 7.05 ± 0.41<sup>b</sup> | 4.78 9.33 |
| Control    | 10 | 61.15 ± 2.01<sup>c</sup> | 51.28 74.13 |

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