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Research Article

The Efficacy of Thymol and Oxalic Acid in Bee Cake Against Bee Mite (*Varroa destructor* Anderson&Trueman) in Honey Bee (*Apis mellifera* L.) Colonies

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Abstract

In the present study, two organic compounds (thymol crystals and oxalic acid crystals in bee cake) with pollen supplement feeding were used to treat colonies against *Varroa destructor*. The organic compounds were applied as follows: (1) 10 g of powdered thymol was added to the bee cake prepared with fat- free soy flour, pollen and 1:1 sugar syrup (TBC), (2) 4 g of oxalic acid was prepared using the same cake recipe described above (OBC), (3) untreated colony (CC). 60 g of cakes from both treatments applied on the top of the frames in colonies. Treatments were carried out in three applications and mite fall were counted weekly during the experiment. The best result was obtained with thymol-bee cake (92.85%). The efficacy of oxalic acid treatment was recorded as 66.72%. There was no significant difference in the level of parasitization inside cells, bee population and brood area among treatments. However there was a significant reduction in mite infestation on adult bee with thymol-bee cake. This result indicates that thymol added to the bee cake is an effective, easy and safe alternative fall treatment for varroa mites.

Keywords: *Varroa destructor, Honey bee, Thymol, Oxalic acid, efficiency, Bee cake*

Bal Arısı Kolonilerinde (*Apis mellifera* L.) Arı Akarı (*Varroa destructor* Anderson&Trueman) İle Mücadelede Arı Kekine Katılan Thymol ve Oksalik Asidin Etkisi

Özet

Çalışmada, *Varroa destructor* akarına karşı polen içerikli iki organik bileşik (thymol kristal ve oksalik asit kristal içerikli arı keki) kullanılmıştır. Organik bileşikler aşağıdaki gibi uygulanmıştır : (1) 10 g thymol yağsız soya unu, polen ve 1:1 şeker şurubu ile hazırlanmış arı kekine katılmıştır (TBC), (2) 4 g oksalik asit yukarıda bahsedilen aynı kek tarifi kullanılarak hazırlanmıştır (OBC), (3) Muameleye tabi tutulmamış koloniler (CC). Her iki uygulamadan hazırlanan 60 g kek kolonilerde çerçevelerin üzerine yerleştirilmiştir. Tedaviler üç uygulamada gerçekleştirilmiş ve akar düşüşü deneme süresince haftalık sayılmıştır. En iyi sonuç (%92.85), thymol-arı kekinde elde edilmiştir. Oksalik asidin etkinliği %66.72 olarak kaydedilmiştir. Petek gözlerde akar oranı, arı populasyonu ve yavrulu alan bakımından gruplar arasındaki fark önemsiz bulunmuştur. Ancak, thymol-arı keki grubunda ergin arılar üzerinde akar bulaşıklığında önemli derecede azalma tespit edilmiştir. Bu sonuçlar, arı kekine katılan thymolun sonbaharda varroa akarına karşı etkili, kolay ve güvenilir alternatif bir mücadele yöntemi olabileceğini göstermektedir.

Anahtar sözcükler: *Varroa destructor, Bal arısı, Thymol, Oksalik asit, Etkinlik, Arı keki*

INTRODUCTION

The mite *Varroa destructor,* a parasite of *Apis mellifera,* has to be controlled by the regular use of acaricides in order to maintain honeybee colonies [1]. Synthetic miticides such as coumaphos, amitraz, fluvalanite and flumethrin have the disadvantage of leaving residues in honey and wax, being a hazard to handle, having some level of toxicity to bees and humans and also because mites quickly develop resistance to their active ingredient [2]. On the other hand, the use of organic compounds such as thymol and oxalic acid that does not show any toxic effects on human and honey bees can be very effective solution in the fight against varroa mite. Thymol is a

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phenolic monoterpene extracted from garden thyme *(Thymus vulgaris)* and toxic to varroa mites [3]. The efficacy of thymol has been tested in previous studies and recommended that the product is a well-known effective compound against the varroa infection which can be used in place of chemical treatments such as coumaphos and amitraz [4,5]. The uses of organic substances containing thymol and oxalic acid have been studied extensively in controlling the varroa mite [6].

Therapeutic medicines may be incorporated in bee cake for treatment and control of bee disease. However, medicines in cake or syrup should not be given to colonies during a honey flow or 5 weeks prior to honey flow [7].

In our experiment, we evaluated the efficacy of thymol and oxalic acid added to bee cake to control *V. destructor* infestations in honey bee colonies during early fall in eastern Turkey.

MATERIAL and METHODS

A total of 24 honeybee colonies were placed in Langstroth hives and headed by Caucasian queen bees raised in Erzurum. Before the trial, colonies were initially equalized to contain a similar amount of brood and bee population and managed in the same way until the miticide trails initiated.

The natural mite mortality was monitored four times, between July 22 and August 21, prior to the treatments. The level of infestation of the experimental colonies was determined using sticky bottomboards three weeks before starting the miticide trials. The colonies were then randomly divided into three groups and treated for the varroa mites.

Treatments began on September 9, 2013. For thymol treatment, 10 g of powdered thymol was added to the bee cake using 20 g of pollen, 1.5 g of fat-free soy flour and 100 ml of sugar syrup (one part water one part sucrose) (TBC). Sixty grams of small cake was applied over the combs of brood chamber. Treatment 2 consisted of 4 g of oxalic acid was added to the bee cake using same mixture described above (OBC). All treatments applied weekly during 3 occasions. Cakes were replaced with new cakes every week. Control colonies (CC) received only 60 gram of miticide free bee cake.

To estimate the mite infestation levels of sealed worker brood, 5×5 cm pieces of comb containing brood were cut with a knife and put in plastic bags. Before and after the treatment, the number of mite from treated and control hives was counted in 300 cells/brood sample.

Adult bee infestation level was determined using a wire net. Approximately 200 workers were collected from each hive in small plastic jars which were filled with water and detergent. The mixture was shaken couple minutes while then adult bees were separated from varroa mite using double layer filter honey sieve under water stream. Mites and bees were counted to establish an infestation rate (number of mites/number of bees x 100) [8].

The capped brood area in each colony was estimated with digital photography. A picture of each side of brood frames was taken with a digital camera. The capped brood area from the digital pictures of each colony was measured to the nearest cm^2 using Adobe Photoshop® CS2 9.0 with a computer as per [9].

The degree of effectiveness of the treatments tested was determined by their percentage of control in relation to the synthetic miticide (Perizin®). At the end of the third week of the experimental trials, each colony was treated with two Perizin® treatments according to the instructions of the manufacturer. The percent efficacy of each treatment was calculated by dividing the number of mites that fell during the period of experimental treatment, by the total number of mites (mite drop of experimental and finisher treatment with Perizin®). The resulting figure was then multiplied by 100.

Data on percent efficacy were analyzed by analysis of variance (ANOVA) after (arcsine $\sqrt{y}/100$), arcsine transformation in the case of percentages, to reduce the heterogeneity of the variance. Means were separated applying the Duncan test ($P<0.05$) [10].

RESULTS

Mite levels in colonies were not different among the treatment groups prior to the treatments (34.25±8.0; $F_{2,21} = 0.28$; $P = 0.76$). The miticide efficacy of the TBC method (92.85±0.7%) was different to that of the OBC method (66.72±2.4%) and control (42.14±2.7%) *(Fig. 1)*.

The number of varroa mites fallen during the treatments with thymol cakes per beehive were higher than treatments with oxalic acid cakes *(Table 1)*. There was a significant difference between colonies in weekly mite fall. The lowest mite fall (49.25±4.9) after Perizin application was recorded in the group of TBC.

There was no significantly differences between the experimental groups and control groups regarding the size of sealed brood area ($F_{2,21}$=1.402, P=0.268; $F_{2,21}$=0.039, P=0.962, respectively), bee population ($F_{2,21}$=0.309, P=0.738; $F_{2,21}$=0.516, P=0.604) and the infestation level inside cells ($F_{2,21}$=0.413, P=0.667; $F_{2,21}$=0.970, P=0.395) before and after the treatments *(Table 2)*. However, treatments reduced the infestation compared to control. The infestation rate decreased from 10.20±0.46% to 7.41±0.26% and 9.37±1.04% to 8.12±0.47% for TBC and OBC groups, respectively.

Adult bee infestation rate did not show any difference between groups before the treatment. However, mite infestation decreased significantly from 16.07±0.72% to 7.82±0.70% in TBC group and 15.93±0.67% to 11.15±0.54% in OBC group. The lowest infestation rate (7.82±0.70%) was obtained in TBC group after the treatment *(Table 2)*.

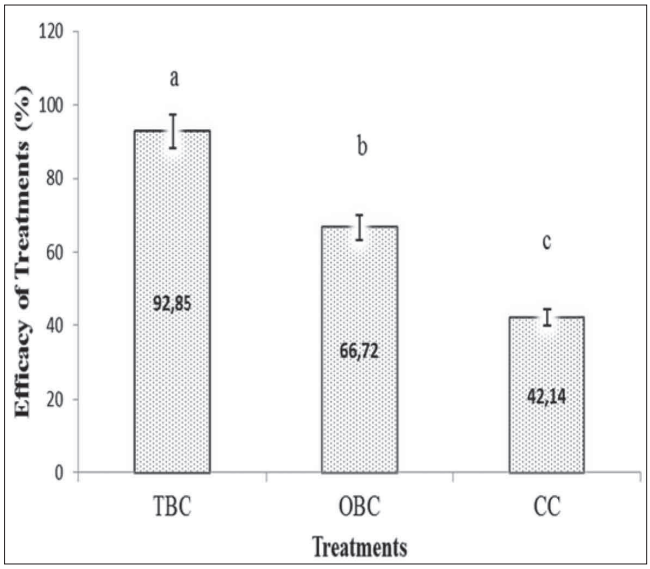

Fig 1. Efficacy of treatments against V. *destructor* infestation
a,b,c Means with different letters are significantly different (P<0.01)

Şekil 1. *Varroa* mücadelesinde kullanılan ilaçların etkinlik derecesi
a,b,c Farklı harfi taşıyan ortalamalar arasındaki fark önemlidir (P<0.01)

DISCUSSION

Treatments effects on varroa mite mortality were significant in colonies. The differences were found significantly important (P<0.01) between thymol and oxalic acid. Thymol bee cakes showed the highest efficacy against varroa control. In our study, the result of mite mortality averaged 92.85% in colonies receiving the thymol-bee cake was higher than the findings, 83.15% and 75.4% reported by different researchers [11,12], respectively. On the other hand, the efficacy of oxalic acid and Apilife-VAR® was recorded as 98.3% and 68.7%, respectively [13]. The results of different authors suggest that application of thymol can be effectively used as an "alternative *V. destructor* control" [5,14].

The efficacy of oxalic acid cake was low compared to the thymol-bee cake treatment. The result of oxalic acid treatments investigated in previous experiments was also evident in our studies [6,15].

The bee population decreased in all treatments at the end of the experiment but no treatment was significantly different than the others. This result was confirmed in

Table 1. *Number of fallen mites during treatments*
Tablo 1. *İlaç uygulamaları sırasında kartonlara dökülen ortalama varroa sayıları*

Treatments	n	N. of Mite Fall per Week			Total Mite Fall	Total Mite Fall with Perizin
		1st week	2nd week	3rd week		
TBC	8	359.75±50.56 a	171.62±19.83 a	111.00±8.82 a	642.38±60.42 a	49.25±4.9 a
OBC	8	150.00±25.93 b	83.62±15.65 b	89.25±6.76 ab	322.88±37.71 b	165.25±9.7 b
CC	8	73.37±15.19 c	52.37±12.53 c	73.50±6.41 b	199.25±78.69 c	293.37±23.6 c

a,b,c Means with different letters are significantly different (P<0.01) measured by One-Way ANOVA followed by Duncan's test for multiple comparisons

Table 2. *Effects of treatments on brood area, bee population, infestation rate inside cells and infestation on adult bee*
Tablo 2. *İlaç uygulamalarının kapalı yavru üretimi, arı varlığı, gözlerdeki bulaşıklık oranı ve ergin arılarda bulaşıklık oranı üzerine etkisi*

Treatments	n	Sealed Brood Area (cm²)		t	Number of Bee Population		t
		BT	AT		BT	AT	
TBC	8	2743.80±154.18 ns	999.49±56.32 ns	ns	40582.6±1373.7 ns	28977.0±743.0 ns	ns
OBC	8	3052.75±86.03	1003.54±25.94	ns	40463.8±1436.8	27862.5±962.6	ns
CC	8	2553.60±323.50	993.23±31.47	ns	41845±1323.0	27119.5±124.9	ns
Treatments	**n**	**Infestation Rate in Brood Cells (%)**		**t**	**Infestation Rate on Bees (%)**		**t**
		BT	**AT**		**BT**	**AT**	
TBC	8	10.20±0.46	7.41±0.26 a	ns	16.06±0.7 ns	7.82±07 a	**
OBC	8	9.37±1.04	8.12±0.47 a	ns	15.93±0.6	11.15±0.5 b	**
CC	8	11.04±0.73	12.75±0.53 b	ns	14.36±0.7	17.01±0.6 c	**

a,b,c Means with different letters are significantly different (P<0.05) measured by One-Way ANOVA followed by Duncan's test for multiple comparisons, ***BT:*** *before treatment,* ***AT:*** *after treatment and ns: not significant,* ***t:*** *comparison between before and after treatments*

previous trials by numerous researchers used different thymol and oxalic acid treatments [16-19]. On the other hand, it was reported that a 4.6% oxalic acid dehydrate solution had negative effects on bee population [19].

Infestation on adult bees decreased using two organic products (TBC and OBC) after the treatments. In a study, oxalic acid treatment reduced infestation from 33.6% to 7.7% [20]. Haggag and El-Badawy [21] reported 89.6%-94.1% reduction in infested brood after the 3rd treatment with thymol, camphor, garlic and thymol+garlic, respectively.

Using thymol-bee cake is inexpensive, easy to use and efficient against varroa mite. Both organic products decreased mite infestation on adult bees and did not cause any damage on colony development.

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