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EFFICACY OF REPEATED TREATMENTS USING OXALIC ACID WITH TRICKLING AND SPRAYING METHODS TO CONTROL VARROA MITES UNDER BROODRIGHT CONDITION OF HONEY BEE COLONIES

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Abstract: This study was carried out in apiary at Assiut region during November, 2007. The efficiency of repeated treatments using oxalic acid (3.2% concentration) with trickling and spraying methods to control varroa mites under brood decrease condition in honey bee colonies was studied. After 24 hours of oxalic acid applications by trickling and spraying, the dead fallen mites were significantly higher in both methods (445.4 and 607.4 mites/colony, respectively) than control (21.2 mites/colony), resulting 20.0 and 27.7 efficiency index, whereas after 48 and 72 hours the results were similar to 24 hours but the levels of fallen mites were low. The first application of oxalic acid by the two tested methods produced significantly more fallen mites (653.0 and 743.2 mites/colony, respectively), inducing 10.8 and 12.4 efficiency index. Similarity, was in both of the second and the third applications by the two methods but were in lowest levels of fallen mites. The cumulative efficiency of the three oxalic acid applications by trickling and spraying methods to control varroa mites in broodright colonies was 94.61 and 94.05% as compared with untreated control colonies. The effectiveness of the trickling and spraying treatments was not significantly different. A non significant difference was observed in number of dead bees in the treated colonies with oxalic acid by both examined methods. It can be recommended to control varroa mites of the beekeepers using the oxalic acid in concentration of 3.2%. Especially, using of trickling method because it was less consuming time and labor intensive yet had equivalent efficiency when compared with the spraying method.

Key words: Honey bee, varroa mite, oxalic acid, trickling method, spraying method

Introduction

Varroa destructor (Anderson and Trueman, 2000) (formerly *Varroa jacobsoni* Oud.) is currently a major pest and obligate ectoprasitic mite of the honey bee, *Apis mellifera* L. Mite females parasitize and feed on haemolymph of both adult bees and brood but develop and reproduce only in brood cells (Martin, 1997). When a young bee emerges from a cell, the mites and their offspring also leave the cell. Subsequently, mite females disperse over adult bees to spend part of their life cycle (Le Conte and Arnold, 1987) and remain on adult bees until they invade new cells. The cycle of transport by bees invasion and reproduction may be repeated several times (Ruijter, 1987). Brood cells are invaded during a certain period preceding cell capping, one and two days for worker and drone brood cells (Boot *et al*., 1992). To find a suitable brood cell, mites move directly from the bee into the selected cell. The mites probably use a signal coming from the larva like heat production or production of volatile chemicals (Boot *et al*., 1994). Varroa mites prefer to invade drone brood cells more than worker brood cells, when they are both available in the colony (Sulimanovic *et al*., 1982 and Schulz, 1984). The infestation rate of drone brood cells was found to be about 12 times higher than that of worker brood cells. However, the distribution of mite over cells depends on the way that the mites select a cell for invasion (Boot *et al*., 1995).

Currently, synthetic acaricides are used for control varroa mites. Acaricide-resistant mites and pesticide residues in honey and wax have appeared in many countries. These problems have led to the development of natural substances for mite control, such as different organic acids and essential oils. Among the organic acids, oxalic acid is registered in many countries. Oxalic acid is extensively used for controlling varroa mites in Europe and Canada due to its high efficacy (>90%) and low risk of hive contamination (Charrière and Imdorf, 2002). It has also low acute toxicity to honey bees and a high acute toxicity to varroa mites (Aliano *et al*., 2006). Oxalic acid is the most effective in broodless colonies because varroa mites in brood cells are not exposed to treatments. During broodless periods, all mites are phoretic on adult bees and vulnerable to treatment. European studies have concentrated on a single application to broodless colonies in early Winter (Schuster and Schürzinger, 2003).

Few investigators have examined the efficacy of multiple treatments when brood is present, and there are no recommendations as to how frequently colonies would need to be treated when brood is present. The objective of the present study was to investigate the efficiency of using the repeated application techniques by trickling and spraying methods of oxalic acid dehydrate to control varroa mites in bee colonies containing a little brood, and to know their effects on bees mortality.

Materials and Methods

The experiments were carried out in an apiary in Assiut region during of November, 2007, because the minimum brood rearing was recorded in this region at the same time (Omar *et al*., 1992).

Preparation of honey bee colonies:

Twenty-four honey bee varroa-infested colonies (10-50% infestation rate) of the first hybrid of Carniolan honey bees, *Apis mellifera* L., in about equal strength (about 5 combs covered with bees), containing little brood were selected for these experiments. Plastic sheets divided into squares were placed on the bottom board of each of the hives in order to record number of fallen mites and dead bees. Wooden frames with wired screans were placed above the sticky sheets to prevent bees from coming in contact with debris.

The colonies were divided into three groups (eight colonies for each), the first group was treated with trickling method of oxalic acid, the second group was treated with spraying and the third one was only treated with a sugar solution (control).

Detection of varroa mites within honey bee colonies:

The detection method used in the present study was performed according to the new technique (powdered-sugar shake) suggested by Ellis (2000) and Macedo and Ellis (2001) as follows: required equipment; - A wide-mouth mason jar with a two-piece lid. Remove the centeral portion of the lid and replace it with # 8-mesh screen. – powdered sugar. -# 8-mesh (3 mm x 3 mm mesh) hardware cloth that will retain the bees while letting powdered sugar to pass through but not varroa mites.

The procedure: Approximately 100 worker bees sampled from near the middle of the hive into the wide-mouth mason jar were brushed. The modified lid was replaced and added a heaping table spoon of powdered sugar through the mesh screen was added. The jar was rolled from side to side to distribute the sugar over all of the bees. Few minutes later and rolled the jar again. The sugar and dislodget mites were poured throu-gh the screen onto cheesecloth. The mites were then separated from the sugar by sifting the sugar through the cloth, leaving the mites on the cloth surface. The bees can then be returned to the colony where their hive mates will groom them clean because the sugar stimulates the bees' grooming behavior. The powdered sugar makes it difficult for the mites to adhere to their host, causing the mites to fall off the bees. This technique works well and separates up to 90% of the mites from the bees. This detection was done at two times, pre- and post-treatments.

The infestation rate of varroa mites was calculated by the following formula (Alloui *et al*., 2002):

$$\textbf{Infestation rate} = \frac{\textbf{Number of varroa}}{\textbf{Number of worker bees}} \times 100$$

Bioassay tests:

In the present study the 3.2% concentration of oxalic acid sugar water solution was used because it is high effective concentration (Fries, 2007). The recipe for making the treatment material (3.2% oxalic acid in a 1:1 sugar solution) as follows: make up a sugar syrup consist of 1 kg sugar in 1 l of water. To this, 75 g of oxalic acid dehydrate was added and mixed well. This will make 1.67 L of treatment material.

Methods of application:

- Trickling method: to trickle oxalic acid, a solution of oxalic acid is applied with a large syringe directly onto adult bees occupying the spaces between the combs. Rademacher and Harz's (2006) found that most researchers recommended a dose of 5 ml per bee space (30-50 ml per hive).

- Spraying method: a solution of oxalic acid and sugar water is sprayed onto the adult bees on both sides of each comb. The combs were individually removed from their hive for application and adult bees located on the hive walls and bottom board are sprayed as well. The doses of 3-4 ml per comb side were applied (Charriere *et al*., 2004).

- The colonies received three oxalic acid applications (one every week) by trickling and spraying methods during November month.

- The control colonies were treated with sugar solution only.

Efficiency of oxalic acid:

On the sampling times, 24, 48 and 72 hours after treatments, the number of fallen varroa mites and dead bees was recorded then the bees and mites were emptied. The efficiency index of oxalic acid was calculated using the following suggested equation:

$$\textbf{Efficiency index} = \frac{\textbf{N.FMT} - \textbf{N.FMC}}{\textbf{N.FMC}}$$

N.FMT, number of fallen mites by treated substance

N.TMT, number of fallen mites naturally (control)

- The index value ≤ 0.0, means that the treated substance dose not have any effect.

- The index value = 1, means that the fallen mites by treated substance gave two fold compared to control.

- whereas the value = 2, means that the fallen mites by treated substance gave three fold compared to control.

At the end of the experiments, to evaluate the efficiency of treated oxalic acid on varroa mites; the reduction percentages were estimated using the formula of Mulla *et al*. (1971) as follows:

% reduction = 100 – [(C_1/T_1) x (T_2/C_2) x 100]

Where C_1 = pre-treatment population density in control.

C_2 = post-treatment population density in control.

T_1 = pre-treatment population density in treatment.

T_2 = post-treatment population density in treatment.

Statistical analysis:

The statistical analysis was conducted using the SAS general linear models procedure. Differences among means were determined by L.S.D. at $P<0.05$ (SAS Institute, 1990).

Results and Discussion

After 24 hours of the first application of oxalic acid by trickling and spraying methods in broodright colonies, the mean number of fallen mites was significantly higher in both methods (445.4 and 607.4 mites/colony, respecttively) than control (21.2 mites/ colony), resulting 20.0 and 27.7 efficiency index, respectively (Table 1, Fig. 1).

Also, 48 hours after the applications the number of fallen mites was significantly higher in both methods than control. Similarly, 72 hours after the application the number of mites in both methods was significantly higher than control. Also during the first application, the results indicated that the numbers of dead fallen mites were significantly higher after 24 hours than after 48 and 72 hours which exhibited the lowest numbers in both of the application methods.

Table(1): Efficiency index of trickling and spraying treatments of oxalic acid to control varroa mites in little broodright colonies.

Treatments	Efficiency index							
	24 hours after treatment		48 hours after treatment		72 hours after treatment		Total of means	
	Trickling method	Spraying method	Trickling method	Spraying method	Trickling method	Spraying method	Trickling method	Spraying method
First treatment	20.01	27.70	7.6	3.1	1.8	2.8	10.8	12.4
Second treatment	18.8	31.03	2.5	2.2	8.2	4.5	8.1	10.7
Third treatment	3.20	3.10	2.0	2.5	1.4	2.0	2.3	2.6
Grand mean	13.20	18.90	3.8	2.5	3.0	2.9	7.0	8.5

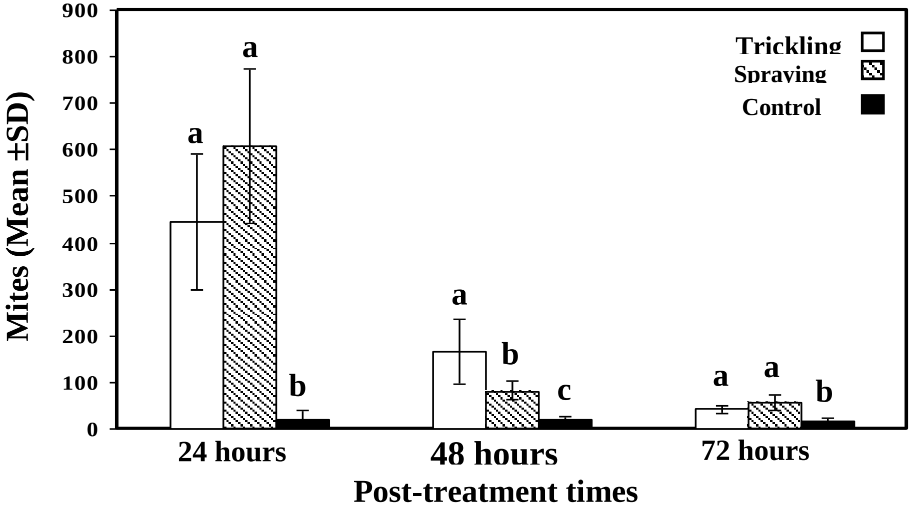

Fig.(1): The mean numbers of fallen varroa mites after the first treatment of oxalic acid using two methods. Treatments followed by the same letters are not significantly different at 0.05 level of probability. Vertical lines denote standard errors.

During the second application of oxalic acid by both methods, the fallen mites were significantly higher in both methods compared to untreated control. Also, the mites were significantly higher after 24 hours than after 84 and 72 hours, which exhibited the lowest numbers of both the treatment methods (Fig. 2).

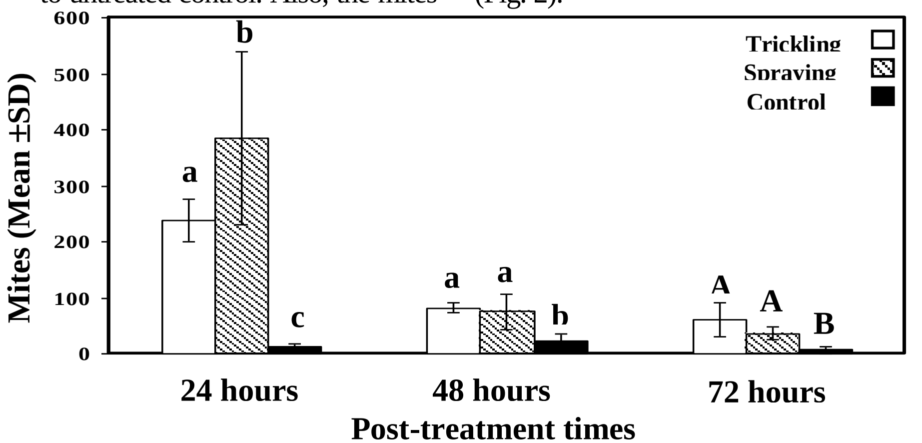

Fig.(2):The mean numbers of fallen varroa mites after the second treatment of oxalic acid using two methods. Treatments followed by the same letters are not significantly different at 0.05 level of probability. Vertical lines denote standard errors.

Similarly, the third application of oxalic acid by the two methods gave significantly more fallen mites than control, and more mites after 24 hours than 48 and 72 hours (Fig., 3).

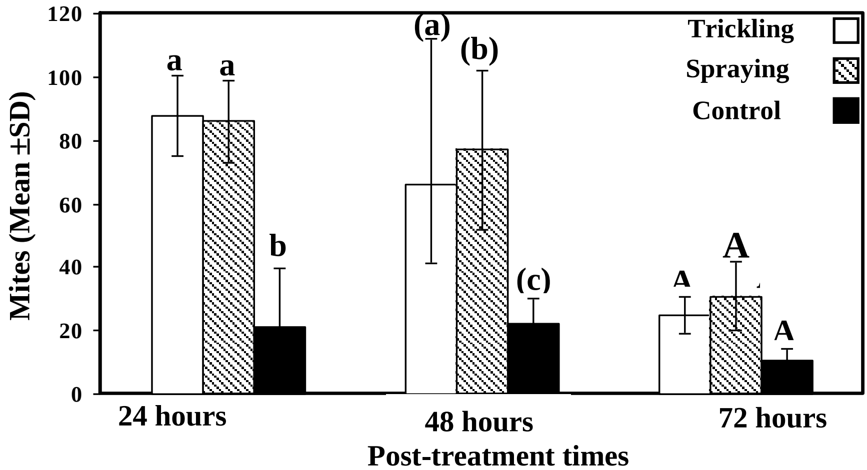

Fig.(3):The mean numbers of fallen varroa mites after the third treatment of oxalic acid using two methods. Treatments followed by the same letters are not significantly different at 0.05 level of probability. Vertical lines denote standard errors.

During all treatments (three applications), after 24 hours for all applications the mean numbers of fallen mites were significantly higher in both trickling (257.4 mites /colony) and spraying (359.3 mites/colony) than control (18.1 mites/colony), resulting 13.2 and 18.9 efficiency index. After 48 and 72 hours the results were similar to those after 24 hours, but were in the lowest levels of fallen varroa mites (Table 1, and Fig., 4).

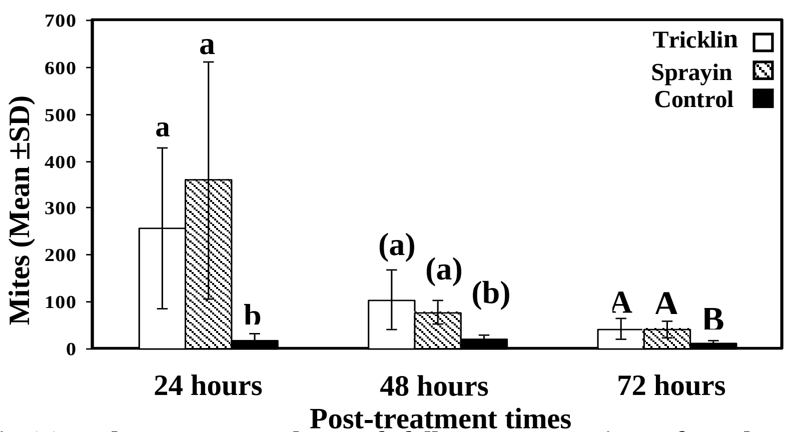

Fig.(4): The mean numbers of fallen varroa mites after the three treatments of oxalic acid using two methods. Treatments followed by the same letters are not significantly different at 0.05 level of probability. Vertical lines denote standard errors.

These are confirmed by Milani (2001) and Aliano (2008) who demonstrated that the oxalic acid has a high acute toxicity to mites in laboratory bioassays 24 hours post-treatment.

In general, the first application of oxalic acid by trickling and spraying produced significantly more fallen mites (653.0 and 743.2 mites/colony, respectively) compared to untreated control (55.4 mites /colony), inducing 10.8 and 12.4 efficiency index. Similarly both of the second and the third applications by the two methods resulted significantly more fallen mites compared to untreated control but were in the lowest levels of fallen mites (Fig., 5).

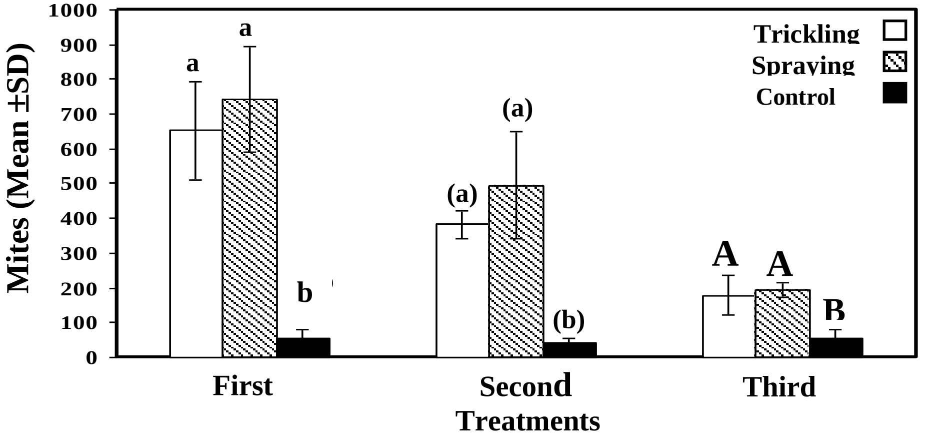

Fig.(5): Total mean numbers of fallen varroa mites during the three treatments of oxalic acid using two methods Treatments followed by the same letters are not significantly different at 0.05 level of probability. Vertical lines denote standard errors.

The first application of oxalic acid in little broodright by trickling and spraying methods produced the highest fallen mites (53.8, 51.9%) in relation to all fallen mites. This finding is in agreement with Bacandritsos *et al*. (2007) and Floris *et al*. (1998) who observed that after four weekly applications of oxalic acid in bee hives in Mediterranean area, the highest fallen mites during the first. While, during the second and the third applications there was a decrease in the dead fallen mites. The highest level of dead fallen mites during the first application, may be due to the high level of foretic mites outside the capped brood during this period. Whereas the level of foretic varroa mites on bees was low during the second and the third applications. Nanetti and Stradi (1997) found that one application of oxalic acid by trickling an oxalic acid sugar solution onto the frames in the colonies provided efficacies ranging from 89.6 to 96.8% depending upon the oxalic acid concentration.

In general, the total mean number of fallen mites in treated colonies by trickling and spraying methods (404.6 and 477.6 mites/ colony, respectively) increased significantly in comparison with the untreated control colonies (50.4 mite/ colony), resulting 7.0 and 8.5 efficiency index, respectively (Table 1 and Fig. 6).

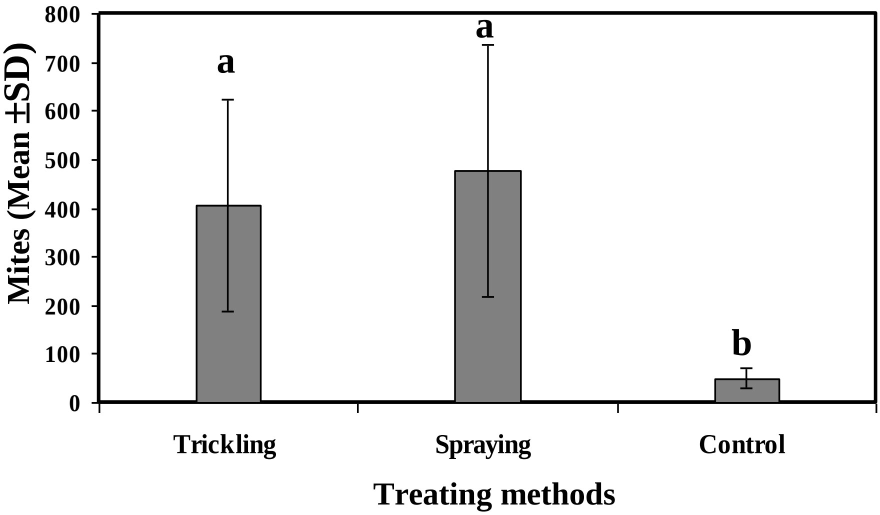

Fig.(6):Total numbers of fallen varroa mites after treating with oxalic acid using two methods Treatments followed by the same letters are not significantly different at 0.05 level of probability. Vertical lines denote standard errors.

The effectiveness of the trickling and spraying treatments was not significantly different. This is confirmed by Aliano (2008), who found similar result.

The pre- and post-treatment varroa infestation levels for all colonies and the percentage reduction in mite infestation 24 days post-treatment were calculated. The trickle treatment reduced mite infestation by 94.61% compared to untreated control colonies, while, the spray treatment reduced varroa infestation by 94.05% when compared to untreated colonies. These findings are in agreement with that of Bahreini (2003) who showed that the dead fallen mites was 99, 95 and 6.8% for spraying, trickling and control, respectively. Fallen mites was not significantly different between spraying and trickling methods.

In the warm climate, the brood is continually present and the broodless period is very short, therefore mite reproduction is high, therefore several treatments may be necessary. Trials with repeated treatments (every seven days for four weeks) were carried out when brood was present in the colonies and led to a reduced mite mortality to 73% in spring and 94% in autumn, respectively (Higes *et al*., 1999). So, the efficiency of oxalic acid treatments is very higher in autumn when there is little brood than those applied to the colonies with brood (Gregorc and Planinc, 2001; 2002).

In contrast, numerous field studies that quantified the efficacy of oxalic acid on broodless colonies had been published in the European literature and reported about 90% efficacy against varroa mites when using either the trickle or spray treatment methods (Imdorf *et al*., 1995; Charrière and Imdorf, 2002; Nanetti *et al*., 2003; Charrière *et al*., 2004 and Rademacher and Harz, 2006).

Using oxalic acid as a method of controlling varroa mite infestations in colonies has been studied extensively (Gregorc and Planinc, 2001; Gregorc and Poklukar, 2003). The efficacy of three oxalic acid treatments in colonies with brood ranged between 39 and 52%, and was 99% in a broodless period. It has been found to be effective on controlling the varroa mite in honey bee colonies in a variety of climatic conditions (Brodsgaard *et al*., 1999 and Nanetti *et al*., 1995). Oxalic acid is most effective when colonies have little or no brood because oxalic acid does not kill mites in sealed brood cells (Schuster and Schüzinger, 2003). Oxalic acid's efficacy was dependant on the concentration and volume applied (Aliano, 2008). In laboratory tests oxalic acid was even more poisonous than the formic and lactic acids to varroa mites (Brodsgaard *et al*., 1997). In field tests Radezki (1994) found very high mite mortality (97.3%) when spraying 3% oxalic acid on adult mite infested bees. Charrière and Imdorf (2002) found that a slightly more concentrated oxalic solution (3.5%) resulted in greater than 95% efficacy. They added that oxalic acid mixed in 1:1 sugar water exhibits greater miticidal effectiveness than solutions with half as much sugar (1:2 sugar water). Further, Milani (2001) indicated that sucrose is synergistic to oxalic acid under laboratory conditions due to sucrose's ability to cause oxalic acid to become more hygroscopic. Perhaps the sugar water solution adheres better to bees, thus increasing mite exposure to oxalic acid. Trophallactic interactions and fumigation did not significantly influence the distribution of oxalic acid. Bee-to-bee contact was the primary route for oxalic acid distribution (Aliano, 2008).

In the present study, a non significant difference was observed in the number of dead bees of the treated colonies with oxalic acid (3.2% concentration) by trickling (8.5 bees/colony) or spraying (9.2 bees/colony) compared to untreated control colonies (7.2 bees/colony). This finding is confirmed by Aliano *et al*. (2006), Bacandritsos *et al*. (2007) and Aliano (2008), they indicated that the oxalic acid has a low acute toxicity to honey bee and a high acute toxicity to varroa mites. Floris *et al*. (1998) reported no effect in bee behaviour and queen activity following four winter oxalic acid applications. No oxalic acid residues are to be expected after repeated field spraying and trickling use of this acid, therefore there are no objective arguments against the registration of these treatment modes for the control of varroa mites. Three oxalic acid treatments have acaricidal effects during the brood period and their residues posess no risk to the honey (Bogdanov *et al*., 2002). Aliano's (2008) data suggested that the three summer treatments with 6% oxalic acid at one week intervals did not have a negative impact on colony health.

The glutathione S-transferase (GST) activity was measured in individual pupae and adult bees from trickled and control colonies. The results showed that 15 days after treatment the GST activity in pupae and adult bees from the trickled colonies was not different from the GST activity from the untreated colonies indicating that the trickling treatment of colonies with oxalic acid does not seem to have an effect on the level of GST activity in pupae or newly emerged adult bees (Brodsgaard *et al*., 1999). Neither the residues of oxalic acid in honey, the GST activity, nor the colony development after spring treatment with either trickling or spraying with oxalic acid seem to indicate any problems. Since oxalic acid is not fat-soluble no residues will build up in the wax in the treated colonies (Imdorf *et al*., 1998). Thus, residues in honey and wax after spring treatment with oxalic acid seems not to be problematic.

In conclusion, during autumn season (November month) the cumulative efficiency of the three oxalic acid applications by trickling and spraying methods to control varroa mites in little broodright colonies was 94.61 and 94.05% compared to untreated control colonies. The highest dead fallen mites occurred 24 hours following treatments. The first application of oxalic acid by both methods resulted the highest dead fallen mites compared to others. The effectiveness of the trickling and spraying treatments was not significantly different. The oxalic acid has a very low toxicity to honey bees, their residues posess no risk to honey and a high acute toxicity to varroa mites. According to the present findings and previous studies, it can be recommended using oxalic acid to control varroa mites by the beekeepers. Especially using of trickling method may be preferable because it was less time and labor intensive yet had equivalent efficacy when compared to the spray method. It took about 1.5 minutes to treat a colony using the trickle method and about 5 minutes to treat a colony using the spray method (Aliano, 2008). Also, using of spraying method may encourage robbing behaviour between bee colonies because are open and it takes more time than trickling method.

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فعالية الاستخدام المتكرر للمعاملات بحامض الأوكساليك بطريقتى التنقيط والرش لمكافحة حلم الفاروا تحت ظروف وجود حضنة فى طوائف نحل العسل

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أجريت هذه الدراسة بمنحل فى منطقة أسيوط خلال شهر نوفمبر 2007م . وذلك لغرض دراسة فعالية الإستخدام المتكرر للمعاملات بحامض الأوكساليك (تركيز 3.2%) بطريقتى التنقيط والرش لمكافحة حلم الفاروا تحت ظروف نقص الحضنة فى طوائف نحل العسل . وقد أوضحت النتائج أنه بعد 24 ساعة من إجراء المعاملة الأولى بحامض الأوكساليك بطريقتى التنقيط والرش ، أن حلم الفاروا الساقط (الميت) ، كان أعلى بصورة معنوية فى كلتا الطريقتين (445.4 ، 607.4 حلم/طائفة على التوالى) مقارنة بالكنترول (21.2 حلم/طائفة) ، مسجلاً 20.0 ، 27.7 دليل فعالية . بينما كانت النتائج بعد 48 و 72 ساعة من المعاملة مشابهة للـ 24 ساعة ولكن كان مستوى العدد الميت من الفاروا أقل بصورة كبيرة . بصفة عامة أحدثت المعاملة الأولى بحامض الأوكساليك بكلتا الطريقتين موتاً لعدد كبير من حلم الفاروا (653.0 ، 743.2 حلم/طائفة على التوالى) ، مسجلة 10.8 ، 12.4 دليل فعالية . وقد أحدثت كلتا المعاملتين الثانية والثالثة نتائجاً مشابهة للمعاملة الأولى ولكن كان مستوى العدد الميت من حلم الفاروا أقل بصورة كبيرة . وكان مقدار نسبة الفعالية الكلية للثلاث معاملات لحامض الأوكساليك بطريقة التنقيط وطريقة الرش لمكافحة حلم الفاروا فى طوائف نحل العسل هو 94.61 ، 94.05% على التوالى مقارنة بالكنترول. وأظهرت النتائج أنه لا توجد فروق معنوية فى كفاءة التأثير بين طريقتى المعاملة (التنقيط والرش) . وأوضحت النتائج أن استخدام حامض الأوكساليك بالطريقتين لمكافحة حلم الفاروا كان شديد السمية بصورة معنوية على الحلم وقليلاً جداً بصورة معنوية فى سميته على نحل العسل. وتوصى الدراسة النحالين باستخدام حامض الأوكساليك بتركيز 3.2% فى مكافحة حلم الفاروا تحت ظروف وجود حضنة قليلة (الخريف) وخصوصاً باستخدام طريقة التنقيط لأنها أسهل فى التنفيذ وتحتاج وقتاً أقل وكفاءتها لا تختلف عن طريقة الرش ، كما أن طريقة الرش يمكن أن تشجع سلوك السرقة بين طوائف نحل العسل نظراً لأنها تستغرق وقتاً أطول فى التنفيذ والخلية مكشوفة .