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EVALUATION OF LEMON JUICE FOR CONTROLLING *Varroa destructor* IN HONEYBEE COLONIES.

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Abstract: The present investigation was carried out in the apiary at Al-Fath location, Assiut Governorate, Upper Egypt, from 8th of December, 2007 to 3rd of January, 2008. The aim of this study was to determine the effectiveness of lemon juice on varroa mites, *Varroa destructor* (Anderson and Trueman) in honeybee colonies, *Apis mellifera* L. with little brood in order to reduce varroa population to tolerable levels. Five concentrations (10%, 25%, 50%, 75% and 100%) of lemon juice (v/v) with sugar syrup 1:1 (w/v) were applied against varroa mites on adult workers honeybee. Five applications of tested concentrations were made to each colony during the treatment period. The percentage of varroa infestation on adult workers, number of fallen dead mites, number of dead bees and the reduction percentage of varroa infestation were determined in the tested colonies. The results showed that, the reduction percentages of varroa were 32.514%, 40.577%, 82.88%, 84.411% and 86.613% observed in the treated colonies with 10%, 25%, 50%, 75% and 100% lemon juice, respectively. The possible use of lemon juice against varroa mite in honeybee colonies as an alternative to routine chemical treatments is discussed. The application of these strategies enables beekeepers to keep the varroa infestation below the damage threshold with reasonable additional labor and, at the same time, it assures high quality bee products.

Key words: honeybee, varroa, control, lemon juice.

Introduction

Varroa destructor (Anderson and Trueman, 2000) formerly named *Varroa jacobsoni* Oudemans is potentially the main parasite of *Apis mellifera* L. and it can cause the collapse of untreated colonies in a few years. Colony collapse is due not only to mite infestation, but also to secondary viral, bacterial and fungal infestations (Hung *et al.*, 1996). Mite control is imperative in order to maintain the population of honeybee colonies in most beekeeping regions around the world.

Several chemical substances were used successfully to control mites, and a wide array of chemicals were highly effective, killing more than 99% of the mites present in infested colonies (Ferrer-Dufol *et al.*, 1991). In recent years, resistance to acaricides has become a major problem in the control of varroa. Increased tolerance to the most widely used synthetic active ingredients has been observed. *Varroa destructor* strains have been reported to be resistant to fluvalinate and flumethrin (Baxter *et al.*, 1998), coumaphos (Spreafico *et al.*, 2001), and to amitraz (Elzen *et al.*, 2000a). Also, the use of acaricides should be minimized in beekeeping because of the residues and their breakdown products in honey and wax (Wallner, 1999).

The problems associated with the use of acaricides proved considerable incentive to develop new treatment strategies and screening for potential acaricides that minimize these problems. Natural products having components with various modes of action might provide effective solution to the problem of varroatosis (Imdorf *et al.*, 1999). These natural products such as essential oils and their components or organic acids, especially formic acid, oxalic acid and citric acid (Mutinelli *et al.*, 1997). It was noticed that, citric acid was less toxic to varroa than oxalic acid (Milani, 2001). Also, grapefruit oil (Elzen *et al.*, 2000b), citronella oil (Krauss *et al.*, 1994) and lemongrass oil (Fathy and Fouly, 1995) were used for controlling varroa mites.

The selection pressure for the resistance against natural acaricides is presently low (Milani, 1999). Accumulation in wax does not occur and residues in honey are small and toxicologically not important (Imdorf *et al.*, 1996). For the beekeepers there is no risk in treating the colonies with organic acids if they apply the saffety measures .

Grapefruit (*Citrus* sp.), was found to cause rapid knockdown of varroa after infested bees were exposed to smoke of burning dried leaves (Eischen and Wilson, 1997). Feeding colonies with coriander's extract reduced the infestation of adult workers and pupae, especially in spring (Shoreit and Hussein, 1994). It was observed that, decrease of varroa mites number of hive debris after spraying colonies with anise, carnation, coriander, cumin, eucalyptus and lemon grass oils. Reduction of varroa in hive debris was about 2.2 times more than those inside brood cells (Hussein *et al.*, 2001). The effect of lemon and orange juices, in laboratory and apiary, were studied by Hussein and Omar (1989).

An efficacy of 39.2% was found after three oxalic acid treatments when the brood was present and 99.4% when the brood

was not present (Gregorc and Planinc, 2001). In Assiut region, Upper Egypt, minimum percentage of monthly workers sealed brood was noticed in November and December (Abdel-Rahman, 2 004). Counting the mites that droped from a colony onto a bottom board is a reliable diagnostic method to evaluate the efficacy of an acaricide treatment (Fries *et al.*, 1991).

This paper presents data of the natural mite fall in non-treated and lemon juice treated colonies with low brood in November and December under conditions of Assiut region to evaluate the efficacy of lemon juice on control varroa mites.

Materials and Methods

This work was carried out in the apiary yard at Al-Fath location, Assiut Governorate, Upper Egypt, during the period from 8th of December, 2007 to 3rd of January, 2008.

Thirty honeybees (*Apis mellifera* L.) colonies were chosen for this work in Assiut. They were divided into six groups (each of five colonies) nearly equal strength.All colonies had moderate varroa mite levels (24.2-27.6%). One of these groups as a control, the five treatments were 10%, 25%, 50% and 75% lemon juice (V/V) with sugar syrup 1:1 (W/V). colonies were treated five times, once every six days by using 5 ml of each treatment per frame of bees (bees filling the inter-space between two frames end to end), spraying directly onto the bees.

The percentage of varroa infestation in the tested colonies before and after treatments, was determined in approximately, 100 living worker bees taking directly from the combs using icing sugar (sugar shake method) (Mark and Cliff, 2001). We calculated the infestation rate by the following formula (Alloui *et al.*, 2002).

$$Tx = \frac{Nv \times 100}{Na}$$

where, Tx : infestation rate; Nv: number of varroa; Na: Number of honeybees.

Hive debris was collected and the fallen dead mites was counted on the bottom board by placing a strong white paper on the hive floor. A wooden and wired (3 mm mesh) frame on the top of the paper prevented bees for coming in contact with debris. On the sampling times, 24, 48 and 72 hours after treatments, the number of mites and dead bees was recorded and the insets were emptied.

At the end of the experiment, reduction percentages was estimated using Henderson and Tilton equation (1955) to evaluate the efficiency of tested lemon juice.

Analysis of variance (ANOVA) was carried out for the obtained data to determine if the

treatments differed from each other or control according to the method of Waller and Duncan (Waller and Duncan, 1969).

Results and Discussion

To help beekeepers select safe mite control alternatives, we evaluated the use of lemon juice at several concentrations to determine their effects on mite and bees and to develop application techniques that would protect hive products from contamination.

The effective of lemon juice on varroa mites were illustrated in Tables (1, 2 and 3). Table (1) shows the mean number of collected mites from hives after spraying with lemon juice. In the colonies treated with several concentrations of lemon juice, the maximum mean number of dead mites was observed after the first treatment then, it was gradually decreased from the first spray to the fifth spray (end of the treatment). These results are supported by the finding of Shoreit and Hussein (1994) who found that, the maximum mean number of dead mites was noticed after the first treatment with coriander extract in both of winter or spring feeding, after that, it was gradually deceased after the second and third treatments. The present results in Table (1) also indicated that the numbers of dead fallen varroa where higher after 24 hours, than after 48 hours, and after 72 hours which exhibited the lowest number.

Throughout the first three sprays, all of the treatments were significantly more effective against varroa mites than the untreated group. The highest concentrations (50%, 75% and 100%) of lemon juice caused highly number of fallen dead mites.

Abd El-Wahab and Ebada (2006) recorded significant differences between the Sour Orange, Lemon grass and Citronella oils in different concentrations (25%, 50% and 100%) and control in the mean percentage of varroa infestation at the second week, third week and fourth week after treatments.

In general, the total grand mean number of fallen mortality varroa mites were 32.56±10.406, 61.6±41.29, 141.84±105.864, 161.6±118.887 and 193.96± 137.478 observed in the treated. colonies with 10%, 25%, 50%, 75% and 100% lemon juice, respectively.

Data in Table (2) recorded that, the total grand mean number of dead adult workers were 12.36±4.424; 11.4±3.792; 11.08±3.509; 10.52±2.763 and 10.24±3.534 noticed in the treated groups with 10%, 25%, 50%, 75% and 100% lemon juice, respectively. Table (2) show that, there was no significant differences between the treatments and control after lemon juice applications.

Table(1): Number of fallen mortality mites following lemon juice applications.

Treatments / No. of doses		Mean ± SE					
		10%	25%	50%	75%	100%	Control
1st spray	24 h	23.6e ±7.702	89.4d ±13.847	247.2c ±24.311	272.2b ±24.737	317.2a ±34.311	11.8f ±4.837
	48 h	9.6e ±3.140	14.8d ±4.304	28.0b ±7.550	25.0c ±4.121	23.2a ±9.701	6.6f ±2.140
	72 h	4.2c ±1.837	7.0b ±2.000	7.6b ±2.548	9.8a ±3.304	9.0a ±3.205	3.8c ±0.839
	Total	37.4e ±6.483	111.2d ±13.114	282.8c ±25.450	307.0b ±24.874	359.4a ±29.370	22.2f ±5.280
2nd spray	24 h	24.0e ±6.581	75.8d ±13.114	191.6c ±25.479	237.4b ±24.555	281.0a ±28.210	16.8f ±5.483
	48 h	12.6c ±4.673	15.6b ±4.817	17.6b ±6.302	17.6b ±4.140	21.2a ±7.304	7.8d ±1.837
	72 h	7.0ab ±1.707	7.4ab ±2.894	6.8b ±2.789	8.8a ±1.304	8.8a ±3.837	5.8b ±2.304
	Total	44.6e ±7.103	98.8d ±12.387	216.0c ±24.772	264.0b ±27.798	311.0a ±33.283	30.4f ±6.894
3rd spray	24 h	18.8d ±4.643	30.4c ±6.140	102.2b ±19.311	109.6b ±18.385	146.2a ±25.044	14.6d ±4.894
	48 h	11.6d ±2.548	13.4c ±3.517	13.6c ±5.140	15.4b ±3.894	16.8a ±6.483	8.6e ±2.548
	72 h	4.8bc ±1.447	5.6b ±1.140	4.4cd ±1.548	6.8a ±2.447	7.6a ±2.548	3.8d ±1.837
	Total	35.2e ±7.101	49.4d ±8.074	120.2c ±23.526	131.8b ±23.106	170.6a ±24.127	27.0f ±4.643
4th spray	24 h	15.0d ±2.703	16.8d ±2.837	42.2c ±9.311	50.8b ±8.106	62.4a ±9.127	10.6d ±3.548
	48 h	6.6d ±1.517	7.2cd ±2.314	8.6ab ±2.548	8.0abc ±2.015	9.2a ±0.873	7.8bcd ±1.502
	72 h	7.0a ±1.807	5.0c ±1.000	6.0b ±2.707	5.2bc ±1.837	7.2a ±2.447	4.8c ±0.947
	Total	28.6c ±5.837	29.0c ±9.225	56.8b ±13.468	64.0b ±17.000	78.8a ±18.955	23.2c ±7.837
5th spray	24 h	7.2c ±2.837	9.8c ±2.837	22.8b ±8.379	27.2ab ±9.311	35.2a ±10.569	7.2c ±1.447
	48 h	5.8b ±2.837	4.8bc ±1.924	5.4bc ±2.548	8.2a ±1.304	9.2a ±3.447	4.0c ±1.707
	72 h	4.0bc ±1.000	5.0ab ±1.707	5.2ab ±1.837	5.8a ±2.643	5.6ab ±1.517	3.2c ±1.304
	Total	17.0c ±6.517	19.6c ±8.130	33.4b ±8.620	41.2ab ±9.497	50.0ab ±12.700	14.6c ±4.302
Grand total		162.8	308.0	709.2	808.0	969.8	117.4
Grand mean		32.56d ±10.406	61.6c ±41.29	141.84b ±105.864	161.6ab ±118.887	193.96a ±137.478	23.48d ±5.934

Means followed by different letters within the same row are significantly different (P<0.05, ANOVA, LSD).

Table(2): Number of dead bees following lemon juice applications.

No. of doses \ Treatments		Mean ± SE					
		10%	25%	50%	75%	100%	Control
1st spray	24 h	4.6a ±0.894	4.2a ±1.304	4.4a ±0.894	4.8a ±0.837	4.2a ±1.643	5.2a ±1.483
	48 h	5.8a ±0.837	5.4ab ±0.548	6.2a ±1.304	4.0b ±1.581	5.6a ±1.140	6.6a ±0.894
	72 h	4.4a ±1.817	4.6a ±3.209	4.2a ±3.633	3.2a ±3.962	1.6a ±1.140	3.4a ±1.342
	Total	14.8a ±1.793	14.2a ±2.311	14.8a ±1.836	12.0b ±1.314	11.4b ±0.915	15.2a ±2.516
2nd spray	24 h	3.2a ±1.095	1.4a ±1.673	1.8a ±1.304	2.2a ±1.304	1.2a ±1.095	1.4a ±2.074
	48 h	4.4a ±1.140	5.0a ±0.000	4.8a ±3.033	4.4a ±2.881	5.0a ±1.000	4.8a ±0.837
	72 h	7.6a ±2.793	5.4ab ±1.140	5.6ab ±0.548	5.4ab ±1.140	4.4b ±1.342	5.4ab ±3.130
	Total	15.2a ±0.895	11.8b ±2.111	12.2b ±1.083	12.0b ±1.000	10.6b ±0.934	11.6b ±2.580
3rd spray	24 h	3.2ab ±1.924	4.4a ±0.894	2.4ab ±1.140	3.4ab ±2.191	2.0b ±1.732	3.0ab ±1.225
	48 h	4.0a ±3.536	3.8a ±2.588	3.2a ±2.280	5.2a ±1.483	4.8a ±2.049	5.6a ±1.673
	72 h	2.0a ±2.121	2.0a ±1.225	2.4a ±2.302	1.2a ±1.789	1.4a ±2.191	2.8a ±0.837
	Total	9.2a ±2.617	10.2a ±1.940	8.0a ±2.323	9.8a ±1.173	8.2a ±2.914	11.4a ±2.123
4th spray	24 h	6.8a ±2.588	5.2ab ±1.304	3.0b ±1.581	5.0ab ±3.391	5.6ab ±2.302	7.4a ±1.140
	48 h	5.0ab ±1.000	4.8ab ±1.924	5.8a ±1.304	4.0ab ±1.225	3.8b ±0.837	5.2ab ±0.837
	72 h	4.6a ±3.130	5.2a ±2.775	4.8a ±3.033	3.8a ±2.168	5.8a ±3.493	4.4a ±1.140
	Total	16.4a ±2.590	15.2a ±1.392	13.6a ±0.149	12.8a ±0.730	15.2a ±2.342	17.0a ±3.120
5th spray	24 h	1.6a ±0.894	1.4a ±1.673	1.6a ±1.517	1.2a ±0.837	1.4a ±1.140	1.2a ±1.304
	48 h	3.4a ±2.302	3.8a ±1.483	4.0a ±3.162	3.0a ±2.000	3.2a ±2.168	3.8a ±0.837
	72 h	1.2a ±1.304	0.4a ±0.548	1.2a ±1.304	1.8a ±0.837	1.2a ±1.095	1.4a ±1.673
	Total	6.2a ±0.932	5.6a ±1.691	6.8a ±1.502	6.0a ±1.420	5.8a ±0.759	6.4a ±1.336
Grand total		61.8	57.0	55.4	52.6	51.2	61.6
Grand mean		12.36a ±4.424	11.4a ±3.792	11.08a ±3.509	10.52ab ±2.763	10.24b ±3.534	12.32a ±4.081

Means followed by different letters within the same row are significantly different ($P<0.05$, ANOVA, LSD).

Smirnov *et al.* (1984) used a plant acaricidal preparation (KAS-81) with sugar syrup for the control of varroa during the whole season (including over wintering) colonies) without harming the bees.

The obtained results in Table (3) show that, the reduction percentages of varroa mites on adult workers honeybee after lemon juice applications were 32.51%, 40.58%, 82.88%, 84.41% and 86.61% observed in the treated colonies with 10%, 25%, 50%, 75% and 100% lemon juice, respectively. Shoreit and Hussein (1994) found that, percent reduction of varroa infestation on adult workers after coriander application was 75.98%. Krauss and Page (1995) stated that, the 50 up to 80% efficiency in varrosis control using garlic, tobacco, walnut, tomato, wormwood, pine and tansey plants. Fathy and Fouly (1995) recorded that, 10 ppm of lemon grass oil caused 44.9% of the reduction percentage of varroa mites infested bee colonies.

Although, the mode of action of lemon juice isn't known but, the obtained results and the efficiency of lemon juice against varroa mites in honeybee colonies may be interpreted as result of the effect of chemical components of lemon juice: citric acid, citral citronellaland limonene.

Table(3): The reduction percentages of varroa mites infestation on adult .workers in the treated colonies with lemon juice.

Infestation rate / Treatments	Before treatment %	After treatment %	Reduction %
10%	27.6	27.2	32.51
25%	24.2	21.0	40.58
50%	25.6	6.4	82.88
75%	24.6	5.6	84.41
100%	26.6	5.2	86.61
Control	25.2	36.8	-

In laboratory assay, almost 73% of the varroa mites fell from adult bees when exposed to fumes of citral. Also, citronellal caused significant knockdown of varroa from exposed bees, when compared to control knockdown. While, limonene showed a limited effect against varroa mites (Elzen *et al.*, 2000).

Citronella oil is more effective repellent to varroa mites and reduced the varroa population in treated colonies to the lowest value (Abd El-Wahab and Ebada, 2006).

Milani (2001) stated that the mortality in treated capsules indicates that, oxalic acid and citric acid have a contact toxic on varroa mites, without excluding other ways of action. Citric acid was less toxic than oxalic acid.

Else, increasing the number of fallen varroa mites in honeybee colonies treated with lemon juice might be elucidated because the activate of the defense behavior mechanisms of honeybee workers against varroa.

Abdel-Rahman (2004) found that, some defense behaviour mechanisms against varroa mites were detected in some races and hybrids of honeybee.

In conclusion, lemon juice and similar materials decrease the overall population of varroa mites in a colony at high concentrations. Using of lemon juice for controlling varroa mites is simple, effective, safe and sheap treatment.

In all cases control of varroa mite using naturally plant products are more recommended than other chemical acaricides to keep the social life of honeybee away from any harmful effect (Dimetry *et al.*, 2005).

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تقييم عصير الليمون في مكافحة طفيل الفاروا فى طوائف نحل العسل

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أجريت هذه الدراسة فى منحل بمركز الفتح بمحافظة أسيوط فى صعيد مصر خلال الفترة من الثامن من ديسمبر 2007 إلى الثالث من يناير 2008 . والهدف من الدراسة هو تقييم فاعلية عصير الليمون فى مكافحة طفيل الفاروا فى طوائف نحل العسل فى وجود مساحة صغيرة من الحضنة بهدف خفض تعداد الفاروا لمستوى معقول . تم تقييم خمس تركيزات من عصير الليمون (10% ، 25%، 50% ، 75% ، 100%) مع محلول سكرى 1:1 وذلك رشاً على شغالات النحل . وتم تكرار الرش خمس مرات للطوائف خلال فترة التجربة . تم تقدير النسبة المئوية للإصابة بالفاروا على الشغالات وكذلك أعداد الفاروا الميتة والنحل الميت على أرضية الخلايا . أيضاً تم حساب نسبة الخفض فى الإصابة بالفاروا نتيجة للمعاملات . لقد أظهرت النتائج أن نسب الخفض فى الإصابة بالفاروا كانت 32.514% ، 40.577% ، 82.88% ، 84.411% ، 86.613% وذلك فى الطوائف المعاملة بعصير الليمون بتركيزات 10% ، 25% ، 50% ، 75% ، 100% على التوالى . لقد درست إمكانية استخدام عصير الليمون فى مكافحة الفاروا كأحد البدائل للكيماويات التقليدية . إن تطبيق مثل هذه الاستراتيجيات البديلة فى المكافحة تمكن النحالين من أن يحافظوا على مستوى الإصابة بالفاروا دون حد الخطر وبجهد معقول ، وفى نفس الوقت تضمن منتجات نحل ذات جودة عالية .