COMBATTING MITE, *VARROA DESTRUCTOR* ANDERSON & TRUEMAN, IN HONEYBEE, *APIS MELLIFERA* LIN., COLONIES BY SOFT CHEMICALS AND/OR AN INTEGRATED PEST MANAGEMENT

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Abstract:

Eleven volatile oils were screened to control *Varroa* mites. These oils were spearmint, thyme, eucalyptus, marjoram, cumin, garlic, basil, orange, geranium, menthol and eugenol. Promising oils (basil, geranium and eugenol) which exhibited noticeable varroacidal activity, in vitro, were selected. In different in vivo tests, three controlling treatments (in winter, spring and autumn seasons) were conducted. Candidate oils were applied separately, added to a pollen supplement or alternatively in an IPM program with formic acid, oxalic acid and queen caging techniques. To evaluate these treatments, mite % infestation on brood and on bees, counts of dropped mites, brood rearing activity (cm^2) and counts of dead bees were recorded. The obtained results showed that tested materials and/or IPM techniques had variable significant varroacidal efficacies in treated colonies compared to untreated ones. Counts of dropped mites were also significantly high. Some adverse effects including reduced brood area, colony disturbance and bee mortality were monitored in some treatments, especially with those had eugenol, which seemed to be harmful to bees. An IPM approach is very much recommended to control *Varroa* mites and/or other bee diseases.

Key Words: *Varroa destructor* – Control – Soft chemicals – Integrated Pest Management.

الملخـــــــص

مكافحة حلم الفاروا فى طوائف نحل العسل بالكيماويات البسيطة بمفردها أو من خلال برنامج مكافحة متكاملة

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أجريت هذه الدراسة **أولا معمليا** على شغالات نحل مصابة بالفاروا لاختبار بعض الزيوت الطيارة المتـــوفرة والآمنة إلى حد كبير فى مكافحة الفاروا وذلك فى محاولة للحد من استخدام المبيدات المعروفة بتأثيرها الـــسام وتلويـــث منتجات النحل نظراً لبقائها فى الشمع فترة طويلة دون حدوث هدم لها. وقد تم اختبار أحد عشر نوعـــا مـــن الزيـــوتً العطرية لتقدير كفاءتها ضد الفاروا وهى زيوت: النعناع البلدى، الزعتر، الكافور، الريحان، البرتقال، الكمون، الثـــوم، البردقوش، العتر البلدى، بالإضافة إلى نوعين من مشتقات الزيوت العطرية هما الإيوجينول والمنتول واختبر خليط من بعض هذه المواد أيضا. وقد وجد أن أفضلها من حيث التأثير على الفاروا هى زيوت العتر البلدى والريحـــان ومـــشتق الإيوجينول، بينما كانت المواد الأخرى أقل تأثيرا. أما فى **الدراسة الحقلية** فقد أجريت عدة تجـــارب علـــى الطوائـــف المصابة لاختبار أفضل المواد التى أعطت كفاءة عالية فى مكافحة الفاروا معملياً لتطبيقها فى صور مختلفة على طوائف النحل المصابة،وقد تم دراسة تأثيرها على معدل الإصابة بالطفيل (على الحضنة والنحل البالغ) قبـــل وبعـــد المعاملـــة

وحساب نسبة الخفض فيها،و معدل تساقط أفراد الطفيل على أرضية الخلية بعد المعاملة،و نشاط الطوائف متمـــثلاً فـــى إنتاج الحضنة،و كذلك التأثير على النحل البالغ بحساب عدد النحل الميت فى أرضية الخلية.

ويمكن تلخيص نتائج هذه التجارب كما يلى:

أولا: المكافحة فى فصل الشتاء: وفيها استخدمت زيوت الريحان والعتر ومشتق الإيوجينول وخليط منها مـــشبعة فـــى ورق كرتون مساحتها 4 x 4 x 0.4 سم لمكافحة الفاروا (عن طريق التبخير) فى طوائف النحل. وقد وجـــد أن هـــذه المواد خفضت نسبة الإصابة فى حضنة الشغالات بمعدلات 64.0٪ ، 21.5٪ ، 35.0٪ ، 57.2٪ وبمعدلات 66.6٪ ، 77.7٪ ، 68.4٪ ، 66.3٪ على النحل البالغ لهذه المواد على الترتيب. وقد سجل معدل تساقط الفاروا بعـــد المعاملـــة بهذه المواد 35.27 ، 42.27 ، 45.93 ، 36.27 فردا/طائفة ، بالمقارنة بـــ 21.80 فرداً / طائفة للغير معاملة أيـــضاً على الترتيب، وقد كان أعلى تساقط لأفراد الطفيل بعد أسبوعين من المعاملة بفارق معنوى بين المعاملات والمقارنـــة. أما بالنسبة للتأثير على تربية الحضنة وعلى النحل فقد وجد أن لهذه الزيوت بعض التأثيرات العكسية التى نـــتج عنهـــا موت بعض الشغالات أو انخفاض مساحة الحضنة وكان مشتق الإيوجينول أكثرها تأثيراً سلبياً على النحل والحضنة.

ثانيا: المكافحة فى فصل الربيع: أجريت هذه التجربة بإضافة زيت الريحان أو العتر إلى مكمل حبوب اللقـــاح وذلـــك للحصول على نشاط عالى للطوائف وفى نفس الوقت لخفض معدل الإصابة بالفاروا (عن طريق التغذية). وقد أوضحت نتائج هذه التجربة أن هاتين المادتين كان لهما تأثير فعال فى خفض نسبة الإصابة بالفاروا فكان معدل الخفض 95.0٪ ، 92.3 ٪ على الحضنة، و 81.8٪، 60.0٪ على النحل البالغ ، على الترتيب. ولم يتضح تأثير مباشر لهاتين المـــادتين على تربية الحضنة إلا أن زيت الريحان أظهر بعض التأثير الطارد للنحل.

ثالثا: المكافحة فى الخريف (المكافحة المتكاملة): أجريت هذه التجربة لوضع برنامج مكافحة متكاملة لطفيـــل الفـــاروا باستخدام أكثر من مادة أو وسيلة حيث تم تقسيم الطوائف إلى مجموعات عوملت بالتقفيص على الملكات مـــع اســـتخدام الأحماض العضوية (الفورميك، الأوكساليك)، الزيوت الطيارة أو استخدمت هذه المواد منفردة أو ثنائية. وأشارت نتائج هذه التجربة إلى أن هذه المعاملات كان لها تأثير فعال فى خفض نسبة الإصابة على الحضنة والنحل البالغ حيـــث بلـــغ معدل الخفض فى الإصـــابة 95.3 ٪، 71.6 ٪، 65.6 ٪، 84.7 ٪، 100 ٪، 79.4 ٪، 87.6 ٪، 82.6 ٪، 92.9٪ على النحل البالغ، أما بالنسبة للحضنة فكانت نسبة الخفض 72.6 ٪، 71.9 ٪، 68.0 ٪، 86.1 ٪، 88.8 ٪، 94.0 ٪، 92.8 ٪، 93.6 ٪، 92.9٪ وذلك فى الطوائف المعاملة بالآتى: (1) المكافحة المتكاملة ،(2) العتر، (3) الريحان ،(4) العتر + حمض الفورميك، (5) العتر + حمض الأوكساليك، (6) الريحان + الفورميك ، (7) الريحان + الأوكـــساليك، (8) الفورميك ، (9) الأوكساليك وأوضحت التجربة أن المعاملة بالعتر + الأوكساليك أعطت أفضل النتائج بينمـــا كـــان أدناها تأثيرا فى خفض الإصابة هو الريحان مقارنة بالمعاملات الأخرى. وقد سجل معدل تساقط الفاروا أعلى متوســـط (433.0 فرداً / طائفة) فى حالة المعاملة بالأوكساليك، بينما سجلت المعاملة بالعتر + الأوكساليك معدلاً أقل بلغ 202.3 فرداً / طائفة. وكان أقل متوسط (6.0 فرداً / طائفة) فى حالة المعاملة بالعتر وذلك بعد أسبوع من المعاملة، وتـــراوح هذا المعدل للمعاملات الأخرى بين 12.7 – 87.0 فرداً / طائفة0 وبالنسبة لتأثير المعاملات على إنتاج الحـــضنة فقـــد ظهر بعض التأثير السلبى بعد المعاملة بالريحان + الأوكساليك وكذا فى بعض المعاملات الأخرى. وبملاحظـــة النحـــل الميت فى قاعدة الخلية بعد المعاملة وجد ارتفاع ملحوظ فى عدده عند استخدام الريحان بعد الأســـبوعين الأول والثـــانى من المعاملة بفارق معنوى بين المعاملات الأخرى أو فى طوائف المقارنة، كما زاد عدد النحل الميت فى حالة استخدام العتر بعد ثلاثة أسابيع، بفارق معنوى أيضاً مع المعاملات الأخرى أو مع الطوائـــف الغيـــر معاملـــة بينمـــا أظهـــرت المعاملات الأخرى تأثيرا أقل.

وفى ضوء هذه النتائج يمكن التوصية بالآتى:-

1. إجراء المكافحة فى فصلى الخريف والشتاء حيث تكون الطوائف فى حالة أفضل لتحمل أى تأثير المواد المستخدمة وكذا لتجنب تلوث منتجات النحل ،وأيضا تكون الحضنة قليلة نسبيا ،وبالتالى يتركز معظم أفراد الطفيل على النحل البالغ، فيمكن الحصول على نتائج جيدة.

2. لا ينصح باستخدام حمض الفورميك أوالأوكساليك فى الخلايا الضعيفة حيث لا تتحمل تأثيرهما، فيمكن استخدام بعض الزيوت الطيارة (مثل زيت العتر البلدى وزيت الريحان) وهى ذات فاعلية متوسطة إلا أنها مواد آمنة إلى حد كبير ورخيصة ومتوفرة. ورغم بعض التأثير السلبى على الطوائف فإنها تستعيد نشاطها من جديد بعد فترة قصيرة.

3. تطبيق هذه المواد ضمن برنامج مكافحة متكاملة يعطى أفضل النتائج ويحد من استخدام المبيدات التى يستعملها النحالون بطريقة عشوائية وبجرعات كبيرة رغم محاذيرها وأضرارها المعروفة وظهور مناعة الطفيل للعديد منها.

Introduction:

The serious ectoparasitic mite, *Varroa destructor* Anderson & Trueman (formerly *V. jacobsoni* Oud.), is a subject of concern to beekeepers worldwide. This mite which feeds on haemolymph of brood and adult bees causes colony disorder, weakness, decreasing brood and deforming immature and mature bees. It also reduces colony ability to pollinate plants (De Jong *et al*. 1984). Infested colony may die or migrate, besides the economical loss of bees and honey production (Needham, 1988). Parasitizing by *Varroa* destroys the mechanical protective barriers of the integument and impairs the immune system of the bees (Glinski, 1991).

In Egypt, the first *Varroa* mite detection was in September 1987 at El-Arish region. By 1989 this mite had become wide spread and by the autumn of 1990 heavy infestations were found in many regions and many apiaries were nearly destroyed. Different pathogens *e.g.* viruses (acute bee paralysis virus, and deformed wing virus) and fungi are probably transferred to bees by *Varroa* (Allen & Ball, 1996). Recently, six bee viruses were detected in bees infested by *Varroa*, so amplifying pathogenisity in honeybees is expected (Tentcheva *et al.* 2004).

To control bee pests and diseases including *Varroa* mites, many scientists and consequently beekeepers are using chemicals. In spite of effectiveness, its use must be constrained and legally recommended otherwise bee products will be contaminated enough to be dangerous for humans (Delaplane, 1997). Besides, the repetition of these pesticides has caused severe problems such as bee toxicity, and increased probability of disease-resistance (Milani, 1995 and Watkins, 1997). For these reasons, recent researchers approached to control this mite by means which relatively safe *e.g.* natural products, especially volatile oils, and/or mechanical and biological trials. So, contributing to *Varroa* control trend this work aimed to minimize *Varroa* mite population by applying cheap, available and mostly safe plant materials, apmlify their effectiveness via an IPM program and observe the impact of these treatments on bees and colony performance.

Materials and Methods:

This work was conducted at the apiary of the Faculty of Agricultue, at Fayoum Governorate, Egypt, during 2000-2001 season as the following:

A. Selected materials:

Nine botanical volatile oils (extracted by water distillation) were selected to control *Varroa* mites. These oils were: spearmint; *Mentha viridis*, thyme; *Thymus vulgaris*, eucalyptus; *Eucalyptus spp.*; basil; *Ocimum basilicum*; orange; *Citrus sp.*, cumin; *Cuminum cyminum*, garlic; *Allium sativum*, marjoram; *Majorana hortensis*, geranium; *Plergonium graveolens* (locally produced) and two fractionated components; eugenol (synthetic from Winlab, England) and menthol (natural from El-Gomhoria Chemicals Co., Cairo, Egypt). Also, a mixture of eugenol, geranium and basil (1 : 1 : 1 v/v) were tested. These oils were kept cool in fridge till every use.

A.1.Testing selected materials as *Varroa* control agents:

A.1.1. Laboratory treatments (screening):

The method of Koeniger & Fuchs (1989), with some modification, was used as the following: 10 alive local hybrid worker bees (*Apis mellifera carnica*) naturally *Varroa*-infested were kept inside a transparent plastic jar (15 cm height x 8 cm diameter). Three jars were replicated for each treatment. Cardboard (2 x 2 x 0.4 cm) was impregnated with 0.1 ml of the test substance and hung inside the jar with fixed wire. A small plastic cup was bored, filled with a sponge containing sugar syrup (2:1w/v) and was put on top of the bored jar to feed the caged bees. Dead bees or mites fallen through a bottom screen mesh onto a sticky paper were counted each day for a period of three days. Control jars had cardboard-free.

A.1.2. Field treatments:

A.1.2.1. Evaporation: Cardboard plates (4 x 4 x 0.4 cm) impregnated with 2 ml of each oil were put on top of the combs.

A.1.2.2. Feeding: To a pollen supplement consisted of 2:1 wheat germ and honey (Mohanny, 1999), 1 ml of oil was mixed well and a cake of 100 g /colony (Langstroth's hives) was given on top of combs and replaced evey 13 days. The treatments were repeated 4 times with weekly interval. Control colonies had no oil treatments. Three colonies were replicated for each treatment.

B. An IPM procedure:

An IPM program and/or essential oils alone or combined with two organic acids was conducted as the following:

B.1. Tested colonies: Thirty naturally *Varroa*-infested colonies were tested and were divided into 10 groups (including control group). Three colonies / group were replicated.

B.2. Treatments and techniques: Group1. IPM: (a) queens were caged in their colonies under hemispherical screen cages, (b) 60% formic acid (10 ml in 7x15x0.4 cm cardboard), (c) basil oil (1ml oil in 2 x 2 x 0.4 cm cardboard), (d) geranium oil (1 ml in cardboard) and (e) 3% oxalic acid (2 ml/comb spray on bees). Seven day intervals after each treatment were left and after three weeks the queens were released. Group 2. Basil oil (1 ml in cardboard). Group 3. Geranium oil (1ml in cardboard). Group 4. Formic acid 60% (10 ml in 7 x 15x 0.4 cm cardboard). Group 5. Oxalic acid 3% (spray). Group 6. Formic + basil. Group 7. Formic + geranium. Group 8. Oxalic + basil. Group 9. Oxalic + geranium. Group10. Control (no treatment). Cardboards were put on top of mid combs.

C. Evaluating efficiency of the tested materials and techniques:

The efficiencies (%) were evaluated according to the modification of Abbott's formula given by Henderson & Tilton (1955) as the following equation:

The reduction rate of infestation = $100 \times 1 - \{ T_a \times C_b\} / \{T_b \times C_a\}$

Where: T_b is % infestation of mite before treatment, T_a is % infestation of mite after treatment, C_b is % infestation of mite before treatment for the control and C_a is % infestation of mite after treatment for the control.

C.1. Mite fall: The counts of dropped mites were recorded after 1, 7, 14, 21 & 30 days of the first application using plastic sheets (51.5 x 36.5 cm) coated with vaseline placed on the hive bottom board.

C.2. Brood measurement: Sealed worker brood areas (SWB) were recorded at 13-day intervals using a plastic sheet divided into square inches. Then the areas were converted into cm^2 by multiplying in 2.54.

C.3. Monitoring infestation: The *Varroa* infestation (%) on bees and on brood was recorded before and after treatments as follows:

C.3.1. On adult bees: About 100 bees were collected, if possible from combs with open brood, and dipped in water to which detergent (washing-up liquid) has been added. The bees were collected in a wire net, and removed after shaking several times. Mites would have fallen off them and found at the bottom of the container (Ritter, 1981). The bees and *Varroa* mites were counted and the infestation percentage (INFP) was calculated using the relation: INFP = No. *Varroa* mites / No. bees x 100

C.3.2. On brood: An area of 5 x 5 cm of SWB in the middle of worker comb was determined. Their cells were scratched and the counts of all stages of *Varroa* female mites in each cell were recorded.

D. Statistical analysis: Data collected were statistically analyzed and the treatment means were compared at 5% probability levels by LSD test, according to the methods given by Snedecor and Cochran (1967).

Results and Discussion:

A. Winter control:

According to the preliminary results, It was found that oils of geranium, basil and eugenol were the most effective *Varroa*-control agents. And it is very important to mention that eugenol had bad effects on bees and brood. These most effective tested materials (by evaporating) were chosen to determine their effectiveness in control and on colony performance.

A.1. Infestation on brood:

The obtained results, Table 1, indicated that all the tested oils reduced % infestation with different rates. The % infestation decreased from 37.33% to 14.00%, 32.67% to 26.67%, 40.00% to 27.00 % and 39.00% to 17.33 % (% reductions in mites after treatment were 64.0%, 21.4%, 35.0% & 57.2%) in colonies treated with basil, geranium, eugenol and a mixture, respectively.

A.2. Infestation on adult bees:

As indicated in Table 1, the % infestation on adult worker bees decreased from 16.00 to 7.53%, 14.67% to 4.60%, 14.07% to 6.27% and 13.50% to 6.40% (reductions were 66.6%, 77.7%, 68.4%& 66.3%) in colonies treated with basil, geranium, eugenol and mixture, respectively. The higher reduction of mites on bees than on brood may be due to the direct exposure of mites on adult bees to the releasing volatile oils meanwhile, mites on sealed brood are exposed to low doses and to slow release. In this respect, Chiesa (1991) found 96.77% reduction in mite population as a consequence of thymol treatment. Abou-Zaid and Ghoniemy (1993), in Egypt, recorded that infestation reduced from 45.7% to 21.2% (61.28 % reduction) and from 38.6% to 28.2% (39.20 % reduction) in colonies treated with wormwood and cumin, respectively. Eshabah *et al*. (1995) found 35% - 45% reduction in infestation when spraying chamomile, thyme and pudding-pipe extracts, while dusting neem and chamomile reduced infestation by 34% - 39%. Allam (1999) recorded 71.8% & 64.3%, 62.5% & 65.0% and 62.6% & 55.35 efficacy for clove, bay and origanum sprayed during Aug.-Sept. in two successive seasons, respectively, while somking eucalyptus, pepper mint and clove oil gave variable range (45.5%-92.2%). Haggag and El-Badawy (1999) reported 89.6% - 94.1% reduction in infested brood after the 3rd treatment with thymol, camphor, garlic and thymol+garlic, respectively. Ibrahim and Shoreit (1999) recorded 20.5%, 35.4%, 24.8% & 37.0% reduction after the 4th dusting with coriander, caraway, chamomile and neem, respectively.

A.3. Mite fall:

The number of fallen mites after treatment averaged 35.27, 42.27, 45.93, 36.27& 21.80 mites/colony in basil, geranium, eugenol, mixture and control colonies, respectively. The highest mite fall was observed after two weeks of treatment being 55.33, 88.00, 80.33, 71.33& 40.33 mites/colony for the previous treatments, respectively with significance. On the other hand, the low mite fall, in general, was observed after one day of treatment (18.33, 19.00, 29.67, 13.33 & 7.33 mites/colony, respectively), and this may be due to the slow effect of tested oils. Averages of mite fall after 1, 3 & 4 weeks were 37.33, 41.67, 42.33, 14.67 & 10.33, then were 51.67, 37.00, 41.33, 39.00 & 21.00 and 13.67, 25.67, 36.00, 43.00 & 30.00 mites/colony, respectively (table1). The mechanism of mite fall is explained by two ways: (1) inhibiting respiration and (2) disrupting between host smell (scent gland) and oils naturally containing some components found in scent gland *e.g.* geraniol, geranic acid, citral, nerol and nerolic acid.

In this respect, the present findings disagree with those of Chiesa (1991) who found high mite fall in the hours immediately following each application influenced by outdoor temperatures. High mite fall was also reported by Egyptian researchers (Abou-Zaid and Ghoniemy, 1993) and this may be due to high intitial infestation. On the other hand, the present findings are, in general, agree with those of Rezk and Gadelhak (1997) who reported that the total cumulative numbers of fallen mites (after a month) were 54.33, 15.67, 10.67& 33.67 and 37.33, 14.0, 16.33 & 30.67 for *Eucalyptus camaldulensis*, *Mentha* sp., *Eugenia aromatica* and *Matricaria chamomilla* in two treated apiaries, respectively.

A.4. Brood rearing activity:

The obtained data, Table 1, showed that sealed worker brood areas (SWB) started 310.67, 322.00, 347.33, 296.00 & 370.00 cm^2 in colonies given basil, geranium, eugenol, mixture and control, rspectively. A light disturbance in brood rearing, after about four weeks in eugenol-treated colonies, was found since SWB averaged 617.33, 758.33, 218.33, 509.00 & 999.00 cm^2 in the same colonies, respectively. It was shown that eugenol was the most material which limited brood rearing activity. After about six weeks the brood build up was normal in treated colonies except in eugenol-treated colonies, which had the lowest brood area compared to other treatments or control where SWB averaged 1009.33, 1226.67, 107.00, 1256.67 & 1046.67 cm^2, respectively.

A.5. Mortality of adult bees:

The average number of dead worker bees averged 1.00, 2.67, 10.00, 0.00 & 1.33 bees/colony for colonies given basil, geranium, eugenol, mixture and control, rspectively (Table 1). The average number of dead bees was high in case of eugenol-treated colonies (with significance) reflecting, probably, its harmful impact on adult bees and also on brood. The highest bee mortality, for eugenol, was observed after one day, 1^{st} , 2^{nd}, 3^{rd} & 4^{th} weeks being 10.00, 16.67, 52.33, 55.33 & 47.33 bees/colony compared to low numbers in other treatments, which showed least bee mortality (range between 0.00 – 5.67 bees/colony) or control being 1.33, 0.67, 1.33, 1.67 & 0.33 bees/colony at the same periods, respectively.

B. Spring control:

To evaluate the effect of basil and geranium oils as a long-term potential *Varroa* control agents, each oil was mixed with a pollen supplement (PS) which, meanwhile, enhancing colony building up.

B.1. Infestation on brood:

Data presented in Table 2 showed that the % infestation decreased from 26.00% to 5.67% and from 22.00% to 1.33% in colonies treated with basil+PS and geranium+PS, with 81.8% & 95.0% reductions in mite infestation, respectively. In this concern, Hassan (1998) reported that the infestation was reduced to 2% after feeding colonies a pollen substitute containing coriander honey and 13.0%, 44.0% & 62.0% for those fed a pollen substitute + eucalyptus honey, sugar solution or unfed, respectively, Sadov (1981) reported that pine essence might be added to a honey+sugar paste, candy or sugar syrup to colonies in winter and early spring increased brood rearing and honey harvest (15%-50%).

B.2. Infestation on adult bees:

The % infestation decreased from 13.13% to 8.77% and from 5.67% to 0.73% in colonies treated with basil+PS and geranium+PS; reduction in mite infestation averaged 60.0% & 92.3%, respectively. In this regard, Hassan (1998) showed that the infestation was reduced to 2% after feeding colonies a pollen substitute containing coriander honey and 8.0%, 25.0% & 33.0% for those fed a pollen substitute+eucalyptus honey, sugar solution or unfed, respectively.

B.3. Mite fall:

Averages of mite fall were 100.67, 69.00 & 65.00 mites/colony for geranium+PS, basil+PS and control, respectively. Also, Hassan (1998) found that mite fall was high in colonies fed a pollen substitute containing coriander or eucalyptus honey compared to those fed sugar solution or unfed.

B.4. Brood rearing activity:

The obtained results (Table 2) indicated that SWB areas started 1058.00, 875.33, 1421.00 cm^2 in colonies fed on basil+PS, geranium +PS and PS, respectively. The averages of SWB areas were 1550.33, 1460.67& 1749.11 cm^2 for the same treatments, respectively, at the end of the test, with 40.36%, 34.15% & 52.56 % increase for the previous treatments and control, respectively without significance. It is worth noting that brood rearing in treated colonies was not highly affected when adding essential oil to PS, except a repellent effect of basil to bees in some cases.

C. Autumn control (IPM):

C-1-Infestation on brood:

Data (table 3) showed that all the tested treatments reduced the % *Varroa* infestation in treated colonies compared to untreated ones. The infestation decreased from 4.77%, 3.33%, 2.33%, 1.33%, 3.00%, 9.33%, 2.33%, 11.67%& 5.33% to 2.33%, 1.67%, 1.33%, 0.33%, 0.60%, 1.00%, 0.30%, 1.33% & 0.67% for IPM, geranium (G), basil (B), geranium+formic (GF), geranium+oxalic (GO), basil+formic (BF), basil+oxalic (BO), formic (F) and oxalic (O), respectively. The % reduction averaged 72.6%, 71.9%, 68.0%, 86.1%, 88.8%, 94.0%, 92.8%, 93.6%& 92.9% for the same treatments, respectively. Regarding the low initial infestation, significant differences were found between treated and untreated colonies.

C.2. Infestation on adult bees:

The obtained results explained that the % infestation decreased from 5.10%, 5.03%, 2.57%, 3.27%, 4.10%, 5.17%, 1.80%, 3.80% & 3.67% to 0.40%, 2.37%, 1.47%, 0.83%, 0.00%, 1.77%, 0.37%, 1.10% & 0.43% (reduction averaged 95.3%, 71.6%, 65.6%, 84.7%, 100.0%, 79.4%, 87.6%, 82.6%& 92.9%) for IPM, G, B, GF, GO, BF, BO, F and O, respectively. Significant differences were found between treated and untreated colonies. Concerning organic acids, formic acid kills mite by inhibiting repiration, but oxalic acid does by acidity, which is yet unexplained.

The present findings are in general agreement with Egyptian authors; Abo-Zaid and Ghoniemy (1992) who reported that oxalic acid treatment reduced infestation from 33.6% to 7.7%. The same authors (1993) reported that infestation was reduced from 51.60% to 7.45% (89.40% reduction) and from 41.82% to 10.90% (79.00% reduction) in two Egyptian Governorates (Qalubia and Fayoum), respectively after four treatments with 60% formic acid. Also, Ghoniemy (1998), in Fayoum, recorded 77.53%, 69.63% & 39.62% reduction with formic acid in cardboard plates in different applications. El-Shaarawy (1999) recorded 84%, 77% & 75% efficacy at Qalubia Gov. and 85%, 80% & 74% at Giza Gov. for different Apiguard® treatments and 60% for formic acid. On the other hand, Mutinelli *et al*.(1996) found variable efficacies of 49.2% - 98.8%, 15.0% - 83.2%, 3.0% - 98.3% & 51.5% - 68.7% for formic acid, lactic acid, oxalic acid and Apilife-VAR®, respectively. Nanetti (1999) showed that 4.2% oxalic acid solution in 60% sucrose resulted in a remarkable effectiveness (80%-92%) against *Varroa* mites in broodless colonies treated in the autumn/winter. Imdorf *et al.* (1999) reported 37%-96% efficacy for formic acid. They attributed this variable range to the method of application, hive type, acid concentration and climatic conditions.

In addition, Allam (1999) suggested the following IPM program for *Varroa* control: 1. Selection for tolerant bees (hybridization between Egyptian honeybee stocks and pure Carniolan). 2.Using natural materials *e.g.* mint, camphor leaves, and a mixture of both in a smoker. 3.Exposing colonies to sunlight, destroying drone brood and strengthen colonies. 4.Using volatile oils when needed during suitable times. Caron (1999) proposed monitoring mite counts, the subsequent using of pesticides when required, reducing of mites entering the hive by trapping on sticky boards, drone brood trapping and the use of resistant bee stocks. Sammataro *et al.* (2000) tested mite-tolerant queen lines, screens prevent fallen mites from climbing back on to comb, an essential oil, EO, (thymol mixture), and combinations of queens and EO, queens and screens, and screens and EO. They found that EO caused a significant quick drop of mites, while the queen/screen combination had the lowest mite drop. Also, they added that colonies located in open field had significantly fewer mites than sheltered ones. Rice and Winston (2001) compared an IPM treatment (thymol + modified bottom boards + hygienic queens) to Apistan. They showed that all of the systems maintained mite populations to levels below an economically important threshold.

C.3. Mite fall:

The mite fall at the end of the treatment averaged 24.33, 20.31, 28.33, 44.31, 49.13, 46.43, 27.37, 35.22, 100.41& 15.38 mites/colony for IPM, G, B, GF, GO, BF, BO, F and O, respectively. The highest mite fall averaged 433.0 mites/colony while the lowest averaged 6.0 mites/colony after one week for O and G, respectively, while GO averaged 202.3 mites/colony with significant difference. The other treatments showed variable mite fall (ranged between 12.7 - 87.0 mites/colony) also after one week treatment. Egyptian authors reported gradual mite fall after treating colonies with organic acids or volatile oils and recorded variable ranges (Ghoniemy and Abo-Zaid, 1993, Ghoniemy, 1998 & El-Shaarawy, 1999).

C.4. Brood rearing activity:

Results presented in Table 3 indicated that SWB areas started 1281.33, 1115.67, 1071.00, 1420.33, 804.67, 1534.67, 466.33, 715.67, 608.00 & 823.00 cm^2 in IPM, G, B, GF, GO, BF, BO, F, O and (C) colonies, respectively. At the end of the test average SWB areas were 482.04, 399.33, 455.96, 426.88, 400.70, 338.38, 273.54, 309.17, 419.96 & 363.38 cm^2 for the same treatments, respectively. The decrease in SWB in case of formic or oxalic may be due to their noticeable adverse effect on brood rearing and may be due, also, to combination between organic acids and oils. Also, average SWB area recorded 0.00 cm^2 twice in the 3rd and 4th measurements in IPM-treated colonies and that is due to caging bee queens for 21 days to minimize mite population by depriving female mites of reproducing in brood cells, but this procedure obviously caused queen damage or loss in some colonies. In this concern, many researchers reported low brood area after treating with hard chemicals or organic acids (Alekseenok & Shutov, 1986 and Schulz, 1993).

C.5. Mortality of adult bees:

Results in table, 3 showed that dead bees averaged 0.85, 2.48, 5.89, 0.77, 1.04, 0.29, 0.59, 1.85, 1.37& 1.29 bees/ colony for IPM, G, B, GF, GO, BF, BO, F, O and C treatments, respectively. The highest average number of dead bees was noticed in colonies treated with B being 4.0 & 22.0 bees/colony after one and two weeks, respectively with significant difference with other treatments or control. After three weeks, the G treatment showed higher bee mortality (15.0 bees/colony) than other treatments or control during the period of experiment.

In this regard, these findings are in general agreement with those of Alekseenok and Shutov (1986) who found that the number of dead bees were 1.8, 4.1& 5.4 for colonies treated with KAS-81(a mixture containing volatiles), Folbex and control, respectively. Comparing KAS-81 to formic acid, the number of dead bees were 1.4 & 1.9, respectively. Moosbeckhofer (1993) mentioned that colonies were adversely affected and produced 20% less honey than control, besides restless and disoriented bess because of the very strong smell of the materials used which masked the colony smell. He added that 50% of bees had died by the end of winter compared to 10.7% in colonies treated with pyrethroid strips. Schulz (1993) stated that brood was damaged in 37 colonies given Apilife-VAR®.

Also, many researchers recorded some adverse effects on bees after treating essential oils or their components; Lensky *et al*. (1996) found that the use of pure origanum oil during summer was harmful to the bees, and 30% thymol was also harmful depending on dose and ambient temperature. This was found also by Chiesa (1991) and Gal *et al*. (1992). Mattila and Otis (1999) showed that honey production was reduced by 30% during the Apiguard® treatment. On contrast, Mutinelli *et al.* (1996) reported low or absent bee mortality in all tests of formic acid, lactic acid or Apilife-VAR®, and also El-Shaarawy (1999) claimed that honey yield increased after colonies treated with Apiguard® or formic acid.

Conclusion and Recommendation:

The use of organic acids (*e.g.* formic and oxalic acids) and/or essential oils (*e.g.* geranium and basil) combined or through an IPM program to control *Varroa* mites is economically recommended. These materials are inexpensive, labor-efficient and relatively less toxic compared to very expensive and toxic acaricides, randomly used in over doses, by beekeepers. It is agreed that there is no danger of contamination if the treatment is carried out, properly, outside the period of production or storage of honey in the hive. In spite of reduced brood rearing in some cases, colonies could restore their normal strength gradually again. It is worth mentioning that formic acid should not be conducted with weak colonies, however the present results showed that such colonies are severely damaged and could be collapsed.

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