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EFFICACY OF SOME PLANT EXTRACTS AGAINST *Varroa destructor* AND THEIR SIDE EFFECT ON HONEYBEE COLONIES

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ABSTRACT: The present investigation was carried out in the apiary of Faculty of Environmental Agricultural Sciences, Arish University, North Sinai Governorate, Egypt, during 2018/2019 season to study the effect of some plant extracts *i.e.* thyme (*Thymus vulgaris* L.) family Lamiaceae, castor (*Ricinus communis* L.) family Euphorbiaceae, common horehound (*Marrubium vulgare* L.) family Lamiaceae and yellow nutsedge (*Cyperus esculentus* L.) family Cyperaceae at different concentrations and added to sucrose syrup which offered to honeybee colonies on the progress of Varroa mite and brood rearing activity of the test colonies. The bees were sprayed with a solution of 100 mg (dry weight) plant extract diluted in 10 ml sugar syrup. Numbers of dead fallen mites and bees were recorded daily. Results revealed that the extracts of thyme (*Thymus vulgaris* L.) and castor (*Ricinus communis* L.) were lethal for the mite, whereas the other plant extract showed low effect. High concentrations of *T. vulgaris* and *R. communis* (20%) were effective against both mite and bees. Thus, the most promising extract, with minimal insecticidal and maximaml acaricidal activities were from *T. vulgaris* and *R. communis*.

Key words: Plant extract, honey bees, *Varroa destructor*, natural acaricide.

INTRODUCTION

Honey bees (*Apis mellifera*) are of great importance for humanity, not only for their direct products such as honey, pollen, royal jelly and propolis, but also for their important role in pollination of field and horticultural crops, which helps to improve quantity and quality of crops production (Yousif-Khalil and Shalaby, 1992; Moreno, 2016). In spite of the growing interest and demand for honey bee by-products, there has been a decreasing in hives number in some countries in the last decades and many studies have been and still carried out to find the causes leading to this imbalance (FAOSTAT, 2015). This represents an ecological challenge which also contributes to the economic losses held by the loss of honey bee individuals themselves. Different factors have been explored in order to discover a cause for this tendency, including honey bee pests and infections, climate change and pesticides.

There is widespread concern about the worldwide decline in the abundance of *A. mellifera* (Baily, 1982; Qayyoun *et al.*, 2013; Topal *et al.*, 2019). The ectoparasitic Varroa mite (*Varroa destructor*) is considered the most serious pest affecting honey bee *Apis mellifera* L. and plays a central role to honey bee losses and it is the most threatening agent of its colonies throughout the world (Fries and Hansen, 1993; Anderson and Trueman, 2000; Anderson, 2000). This mite completes all its life cycle inside the beehives, attaches to the body of bee adults, pupae and larvae (Alaux *et al.*, 2009; Dahle, 2010), sucking the haemolymph of the pupae and adult bees (Ruffinengo *et al.*, 2007), and reduce the water content and the total body weight thus lowering the protein and carbohydrate content and finally causing the death of the bees (Bowen-Walker, Gunn, 2001; Rahimi *et al.*, 2014). Varroa also increases the

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concentration of a series of viruses and then viruses transferred between the bees via the mite (Ball, 1985; Shimanuki *et al.*, 1994; Garedew *et al.*, 2004). Without periodic treatment, most of the honey bee colonies in temperate climates would collapse within a 2-3 years period (Fries *et al.*, 1994; Nordstrom, 2003; De la Rua *et al.*, 2009; Rosenkranz *et al.*, 2010).

Nowadays, researchers tend to find safer methods to control *Varroa* mite. Including the use plant extracts, that could replace chemical acaricides in the control of mite (Kim *et al.*, 2005; Ghasemi *et al.*, 2010). Compared to chemical pesticides, plant extracts are more compatible with ecosystems, less toxic to living organisms, and leave no persistent killer substances in nature (Liu *et al.*, 2005; Georges *et al.*, 2007). However, more studies are needed to confirm such properties (Wandscheer *et al.*, 2004; Akbarinia and Mirza, 2008; Ghasemi *et al.*, 2010). In Egypt, El-Nagar *et al.* (2019) evaluated the effect of some botanicals-based food mixtures added to sucrose serum and offered to honeybee colonies on the development of *Varroa* infestation. They concluded that the food mixture composed of lemon juice + garlic extract reduced significantly the rate of *Varroa* infestation as compared to the control and the mixtures composed of thyme extract + orange juice that reduced the rate of *Varroa* infestation and showed the least brood rearing activity, being lower than the control.

The purpose of this study was to evaluate the efficiency of some plant extracts on honey bees infested with *Varroa* mite and to investigate their side effect on honey bee colonies.

MATERIALS AND METHODS

Apiary and Colony Preparation

The present investigation was carried out in the apiary of Faculty of Environmental Agricultural Sciences, Arish University, North Sinai Governorate, Egypt, during 2018/2019 season to study the effect of some plant extracts *i.e.* Thyme (*Thymus vulgaris* L.), castor (*Ricinus communis* L.), common horehound (*Marrubium vulgare* L.) and yellow nutsedge (*Cyperus esculentus* L.) at different concentrations to honeybee colonies on the progress of *Varroa* mite infestation, brood rearing activity of the test colonies.

Plant Material and Extraction

The fresh whole plant materials of thyme (*Thymus vulgaris* L.) family Lamiaceae, seeds and leaves of castor (*Ricinus communis* L.) family Euphorbiaceae, aerial parts of common horehound (*Marrubium vulgare* L.) family Lamiaceae and tubers of yellow nutsedge (*Cyperus esculentus* L.) family Cyperaceae were collected from Sinai Peninsula, Egypt, during winter season November 2018 to February 2019, then washed and air-dried in glass greenhouse.

Selected plant materials were subjected to water extraction according to Al-Ghzawi *et al.* (2008) method in the Environmental Protection Dept. Dried plants were grounded to powder using an electric grinder with a 2 mm diameter mesh. Four plant extracts were thyme (*T. vulgaris* L.), castor (*R. communis* L.), common horehound (*M. vulgare* L.) and yellow nutsedge (*C. esculentus* L.) were tested to control *Varroa* mite, extracted by 95% ethanol. Each finely powdered of sample *i.e.* 100, 150 and 200 g were placed in a glass flask (2L) and extracted by cold percolation with 95% ethanol, then the mixture was filtered through filter paper (Whatman No. 2). The final concentrations of the prepared *T. vulgaris*, *R. communis*, *M. vulgare* and *C. esculentus* were 10, 15 and 20% as total solids. The amount of obtained extracts were preserved in sterile dark bottles (500 ml) in a cool environment (4°C) until used. The main phyto-chemical constituents of the tested plant extract as reported in the literature are presented in Table 1.

Investigating the Efficiency of Plant Extracts in Controlling *Varroa* mite

Experimental design

Experiment was performed according to Shimanuki and Knox (2000) method with four plant extracts (groups) with three concentrations of each plant (10, 15 and 20%). Three replicates (honeybee colonies) were considered for each treatment as well as control treatment in a randomized complete design (RCD). Honeybee (*Apis mellifera* L.) colonies were nearly equal in strength and headed by mated sister queens. Such colonies suffered nearly the same rate of *Varroa* mite infestation. The experimental colonies were randomly distributed and divided

Table 1. Main phyto-constituents, part used and harvested areas of investigated Sinai's Flora extracts

Tested plant	Thyme (<i>Thymus vulgaris</i> L.)	Castor (<i>Ricinus communis</i> L.)	Common horehound (<i>Marrubium vulgare</i> L.)	Yellow nutsedge (<i>Cyperus esculentus</i> L.)
Part Used	Whole plant	Seeds + leaves	Aerial parts	Tubers
Harvested areas	St. Catherine - South Sinai	Valley of El-Arish	El-Arish	South Sinai
Main constituents	<ul style="list-style-type: none"> - Thymol - γ-Terpinene - <i>p</i>-Cymene - α-Terpinene - Carvacrol - 2,4-Dymethyl-2,4-heptadiene 	<ul style="list-style-type: none"> - Ricinine - Ndemethylricinine - Glycosides kaempferol-3-O-β-D-Xylopyranoside - Kaempferol-3-O-βD-glucopyranoside - Quercetin-3-O-β-D-xylopyranoside - Kaempferol-3-O-βrutinoside - Quercetin-3-O-β-rutinoside²⁰ - Monoterpenoids (1, 8-cineole, camphor and α-pinene) - Asesquiterpenoid (β-caryophyllene) - Gallic acid, quercetin and gentisic acid - Rutin and epicatechin - Ellagic acid. 	<ul style="list-style-type: none"> - Germacrene D - β-caryophyllene - β-bisabolene - Bicyclogermacrene - Carvacrol - Thymol - Carvyl acetate - β-phellandrene - γ-cadinene. 	<ul style="list-style-type: none"> - 12-methyl cyprot-3-en-2-one-13-oic acid - <i>n</i>-dotriacontan-15-one and <i>n</i>-tetracontan-7-one - <i>n</i>-pentadecanyl octadec-9 - 12- dienoate (<i>n</i>-pentadecanyl linoleate) - <i>n</i>-hexadecanyl linoleate and <i>n</i>-hexadecanyl oleate - <i>n</i>-pentacos-13-enyl octadec-9-enoate - 5,22-dien-3β-olyl <i>n</i>-dodecanoate - Stigmast-5, 22-dien-3β-olyl <i>n</i>-tetradecanoate - β-sitosterol-3β-O-glucoside - Lupenyl 3β-O-arabinpyranosyl 2'-oleate.
Reference	Porte and Godoy (2008)	Kumar (2017); Jena and Gupta (2012)	Shaheen <i>et al.</i> (2017) and Benalia (2019)	Sultana <i>et al.</i> (2017)

into four main groups. Three groups were sprayed with plant extracts at three concentrations and dissolved in sucrose syrup, whereas the fourth group was sprayed with syrup only to serve as control. The test colonies were fed regularly on sucrose syrup (1:1) when needed.

Counting the number of fallen *Varroa* mites and dead bees

Numbers of fallen *Varroa* mites and dead bees were counted after 1, 15 and 30 days of the application using plastic sheets (51.5 × 36.5 cm) coated with vaseline that placed on the hive bottom board and the plastic sheets were covered with screen mesh to allow fallen mites to reach the sticky sheet. The plastic sheets were replaced with new ones at the end of each interval.

Evaluating the rate of *Varroa* mites infestation (%)

A randomized samples of hundred adult workers was picked up from each experimental colony. Bee sample was slightly chilled in freeze then placed in a glass funnel that placed on glass flasks sufficient quantity of boiled water was poured on bee sample in the glass funnel, *Varroa* mite fallen in the flask were counted (V). The rate of mite infestation was then calculated according to the following equation:

$$\text{Varroa Infestation (\%)} = \frac{V}{B} \times 100$$

Where:

V= The number of counted *Varroa* mites and
B= The number of infested bees

Varroa mite infestation was measured at the onset the experiment before applying any treatment, then once post each treatment or after a constant time interval post-treatment.

Calculating the reduction in mite infestation (%)

Evaluation of the tested plant extracts at different concentrations was based on the calculated reduction in *Varroa* mite infestation (%) at 15 days according to **Henderson and Tilton (1955)** equation:

$$\text{Reduction of infestation (\%)} = \left(1 - \frac{Ta \times Cb}{Tb \times Ca}\right) \times 100$$

Where:

T = Infestation (%) of treated colonies,

C = Infestation (%) of untreated colonies,

a = After treatment and b = Before treatment.

Statistical Analysis

Data obtained were statistically analyzed using the method of Randomized Complete Design (RCD) according to **Snedecor and Cochran method (1967)**. The data were calculated according to COSTAT computer program (**Anonymous, 2005**), Means comparisons were carried out by Duncan's multiple range test at (0.05) level of significance (**Duncan, 1955**).

RESULTS AND DISCUSSION

Effect of Plant Extracts on Number of Fallen *Varroa destructor* Mites

Data presented in Table 2 indicated that mean numbers of fallen *Varroa* mites in the treated honey bee colonies with the test plant extracts attained 23.97, 17.86, 14.10, 7.05 and 1.41 fallen mites/colony after 1 day of treatment; and 54.80, 34.45, 39.84, 18.30 and 11.88 fallen mites/colony after 15 day of treatment; and 33.74, 14.00, 22.61, 10.41 and 4.75 fallen mites /colony after 30 day of treatment with the extracts of *Thymus vulgaris*, *Marrubium vulgare*, *Ricinus communis* and *Cyperus esculentus* as well as control colonies, respectively, regardless of concentrations applied. The corresponding mean total number of fallen mite reached 37.50, 22.10, 25.52, 11.92 and 6.01 fallen mites/colony. The number of fallen mites/colony is directly proportional to the rate of increase in the concentrations of plant extracts, where the highest significant mean number of fallen mites was (78.26 fallen mites /colony) was recorded with the highest concentration (20%) of *T. vulgaris*, followed by that the same concentration of *R. communis* (60.29 fallen mites /colony). On the contrary, the lower concentrations showed significantly lower numbers of fallen mite. In general, *T. vulgaris* and *R. communis* extracts provide to be the most effective among the test plant extracts. Analysis of data revealed significant differences between plant extracts and concentration tested. It is obvious that all the test extracts were superior over the control in the number of fallen mite. In connection, **Rahimi et al. (2014)** reported that the use of the thyme extract

Table 2. Number of fallen *Varroa destructor* mites after treatment with the four plant extracts at three intervals post treatments

Plant extract	1 day			Mean	15 days			Mean	30 days			Mean	Total average
	C-10%	C-15%	C-20%		C-10%	C-15%	C-20%		C-10%	C-15%	C-20%		
<i>T. vulgaris</i>	7.05 e	28.20 b	36.66 a	23.97 A	38.76 d	47.37 c	78.26 a	54.80 A	26.92 c	34.45 b	39.84 a	33.74 A	37.50
<i>M. vulgare</i>	5.64 ef	19.74 c	28.20 b	17.86 B	27.99 f	32.30 e	43.07 cd	34.45 BC	7.54 h	12.92 f	21.53 d	14.00 C	22.10
<i>R. communis</i>	7.05 e	15.51 cd	19.74 c	14.10 C	23.69 g	35.53 de	60.29 b	39.84 B	14.00 f	17.23 e	36.61 ab	22.61 B	25.52
<i>C. esculentus</i>	2.82 g	7.05 e	11.28 d	7.05 D	11.84 i	17.23 h	25.84 fg	18.30 C	7.54 h	9.69 g	14.00 f	10.41 D	11.92
Control	1.41 h	1.41 h	1.41 h	1.41 E	10.77 j	10.77 j	14.10 j	11.88 D	4.31 i	4.31 i	5.64 i	4.75 E	6.01
Mean	4.79 C	14.38 B	19.46 A		22.61 C	28.64 B	44.31 A		12.06 C	15.72 B	23.52 A		

Means followed by the same letter(s) within each column are not significantly different at the 0.05 level, according to Duncan's multiple range test.

influenced significantly the percentage of mortality of mite in the extract-sprayed treatment and the control treatment and ethanol extract of thyme show that its use in hives was safe without a high risk of mortality for honey bees after spraying. Many authors agree that the particular active ingredient with acaricidal properties in commercial products is thymol (Akyol and Halil Yeninar, 2008; Ramzi *et al.*, 2017). Pure thymol was commonly tested and showed that the mite mortality rate was between 54 and 98% (Chiesa, 1991; Gal *et al.*, 1992; Higes Pascual *et al.*, 1996) and the effectiveness was similar for different application methods (Imdorf *et al.*, 1999).

Effect of Plant Extracts on Infestation of *Varroa destructor* mite to Brood and Adult Bees as well as Percent Reduction in Mite Infestation (%)

Data in Table 3 indicated that brood and bees infestation with *Varroa* mite was affected by the tested extracts, that reduced the infestation with different rates compared to the control. For instance, the mite infestation was reduced to

4.23, 10.22, 6.66 and 12.11% in worker brood and 4.50, 13.42, 8.23 and 15.38% on adult bees with the treatment of *T. vulgaris*, *M. vulgare*, *R. communis* and *C. esculentus* extracts, respectively compared to control (19.45 and 18.11%). The differences were mostly significant, being the most for *T. vulgaris* in brood infestation and in *M. vulgare* in case of adult bees. Moreover, the highest concentration induced the highest effect. Regarding percent reduction in mite infestation, it's clear that the highest percent reduction (83.13 and 80.23% on brood and adult bees respectively) was recorded with *T. vulgaris* extract whereas the least rate of reduction was taken place with *C. esculentus* extract (51.77 and 33.71% on brood and adult bees, respectively).

Effect of Plant Extracts on the Number of Dead Bees

The results in Table 4 showed that, the average numbers of dead worker bees were 1.82, 4.67, 2.16, 6.33 and 7.73 per colony for *T. vulgaris*, *M. vulgare*, *R. communis*, *C. esculentus* extracts and control, respectively. The numbers of dead

Table 3. Infestation of *Varroa destructor* mite to brood and adult bees as well as percent reduction in mite infestation (%) with plant extracts

Plant extract	Pre-treat%-inf.	Infestation to brood (%)			Mean	Pre-treat%-inf.	Infestation to bees (%)			Mean
		C-10%	C-15%	C-20%			C-10%	C-15%	C-20%	
<i>T. vulgaris</i>	20.04	5.62e	4.67 f	2.39 g	4.23D	18.22	6.67f	4.17g	2.67h	4.50D
<i>M. vulgare</i>	18.45	16.33bc	9.33cd	5.00ef	10.22BC	19.31	16.67b	12.83cd	10.75de	13.42BC
<i>R. communis</i>	19.11	8.65d	6.78de	4.56h	6.66C	20.23	11.17d	8.33e	5.18fg	8.23C
<i>C. esculentus</i>	20.09	17.00b	12.67c	6.67de	12.11B	18.56	17.08ab	15.17bc	13.89c	15.38B
Control	20.03	19.45a	19.45a	19.45a	19.45A	19.74	18.11a	18.11a	18.11a	18.11A
Mean		13.41A	10.58AB	7.61B			13.94A	11.72B	10.12C	
Plant extract	Reduction of mites on brood%			Mean	Reduction of mites on bees%			Mean		
<i>T. vulgaris</i>		77.56bc	81.36b	90.46a	83.13A		70.71bcd	81.69b	88.28a	80.23A
<i>M. vulgare</i>		29.18fg	59.53de	78.32bc	55.68C		30.94fg	46.85e	55.46d	44.42C
<i>R. communis</i>		63.79cde	71.62cd	80.91b	72.11B		55.83d	67.06cd	79.52bc	67.47B
<i>C. esculentus</i>		32.30f	49.56e	73.45bcd	51.77C		26.38g	34.61f	40.13ef	33.71D
Mean		45.03C	56.88B	69.09A			42.09B	51.36A	58.00A	

Percent reduction in mite infestation

Means followed by the same letter(s) within each column are not significantly different at the 0.05 level, according to Duncan's multiple range test.

Table 4. Number of dead bees due to plant extracts treatment against *Varroa* mite at different concentrations

Plant extract	No. of dead bees			Mean	
	Con.	C-10%	C-15%		C-20%
<i>T. vulgaris</i>		1.20g	1.65fg	2.60e	1.82E
<i>M. vulgare</i>		3.20d	4.40c	6.40bc	4.67C
<i>R. communis</i>		1.87efg	2.00ef	2.60e	2.16D
<i>C. esculentus</i>		4.40c	6.80bc	7.80ab	6.33B
Control		7.00b	7.60ab	8.60a	7.73A
Mean		3.53C	4.49B	5.60A	

Means followed by the same letter(s) within each column are not significantly different at the 0.05 level, according to Duncan's multiple range test.

worker bees is directly proportional to the rate of increase in the concentrations of plant extracts, where the highest significant mean numbers of dead worker bees was (7.80 numbers of dead worker bees/colony), which was recorded with the highest concentration (20%) of *C. esculentus*, followed by the same extract at 15% concentration (6.80 numbers of dead worker bees/colony) compared to control. On the contrary, the lower concentration (10%) of *T. vulgaris* showed significantly lower number (1.20) of dead worker bees/colony. Generally the highest concentration with the tested plant extracts (20%) cause the highest number of dead bees whereas the least concentration (10%) provide to be more safe to bees. These results are in partial accordance with the findings of **Islam et al. (2016)** who found that the highest total number of dead Varroa fallen mite on the sheet was recorded with thyme. Similarly, **Abd El-Wahab et al. (2012)** found that the highest reduction mite infestation on adult workers was recorded with the highest concentration (100%) of thyme, whereas more than 96% reduction in mite infestation was recorded in brood. In general, the results obtained suggest that aqueous extract of *Thymus vulgaris* and *Ricinus communis* could play an important role in an integrated program management to control the Varroa mite infestation in honey bee colonies.

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فعالية بعض المستخلصات النباتية ضد حلم الفاروا وأثرها الجانبي على طوائف نحل العسل

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أجريت هذه الدراسة بهدف تقييم تأثير المستخلصات المائية لأربعة من النباتات النامية بسيناء والمضافة رشا على طوائف نحل العسل على معدل الإصابة بحلم الفاروا *Varroa destructor*، وكذلك أثارها الجانبية على حياة شغالات نحل العسل خلال الفترة من أكتوبر وحتى نوفمبر من العام 2019 م بمنحل كلية العلوم الزراعية البيئية - جامعة العريش- شمال سيناء - مصر. تم تطبيق المستخلصات المائية لنباتات الزعتر والخروع والريحان وسعد اللذيذ بتركيزات 10 %، و 15 %، و 20% وتم تصميم التجربة بنظام كامل العشوائية في ثلاث مكررات. أظهرت نتائج الدراسة أن أعلى انخفاض في نسبة الإصابة بحلم الفاروا على الحشرات الكاملة نتيجة معاملة الطوائف بمستخلص الزعتر عند تركيز 20%، يليه مستخلص الخروع عند تركيز 20% وذلك مقارنة بباقي المستخلصات الأخرى. كما أوضحت النتائج أن أعلى نسبة انخفاض للإصابة بالحلم في الحضنة والحشرات البالغة من نحل العسل كان للطوائف المعاملة بنبات الزعتر يليها نبات الخروع. بينما أظهرت النتائج أثر هذه المستخلصات على معدل موت الحشرات البالغة لنحل العسل في بعض المعاملات، خاصة تلك التي تحتوي على تركيز عالي من الزعتر والخروع. وخلصت الدراسة إلي أن معاملة الطوائف بمستخلص مائي من الزعتر أو الخروع عند تركيز 20% يمكن استخدامه في علاج طوائف نحل العسل المصابة بحلم الفاروا والحد من خطورته مع أقل تأثير جانبي علي طوائف نحل العسل.

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