Nat. Volatiles & Essent. Oils, 2022; 9(1): 561-574

NVEO
Natural Volatiles &
Essential Oils

Evaluation Of Oils And Extracts Of Some Natural Materials In The Management Of Varroa Jacobsoni Oudemans Mites On Apis Mellifera Honey Bees In Basrah Province

Amira Jassim Mohammed* , **Khaled Abdul Razzaq Fhad**

Department of Plant Protection - College of Agriculture - University of Basrah - Basrah – Iraq.

Abstract

The experiment was conducted in the apiaries of the Bee Project of the Plant Protection Department in the Basra Agriculture Directorate during the month of October. The study aimed to evaluate the effectiveness of some natural materials in reducing Varroa jacobsoni Oudemans infection on Apis mellifera honey bees, which included neem (Azadirachta indica) and thyme. Thymus vulgaris, Eucalyptus obliqua, Rosmarinus officinali, olive, Olea europaea. Those natural materials were used in three ways to control with volatile oils, by placing a leaf saturated with oil at the base of the hive, smoking using the blower, burning the leaves of plants inside it, fumigating bee colonies, using aqueous extracts of natural materials and using them as a direct spray on honey bees, for the purpose of evaluating the most effective methods of managing the pest.The readings were taken after 24, 48 and 72 hours of treatment. The results of the statistical analysis showed that there were significant differences in the percentage rate of Varroa mite precipitation after treatment with aromatic vegetable oils, as it reached the highest average (85.31%) for neem oil treatment after 48 hours of treatment and the lowest percentage of dropping (20.74%) for olive oil treatment after 24 hours of control compared to the control treatment, which amounted to 14.76% after 24 hours of treatment with petroleum jelly only.The fumigation method had a significant effect on the percentage of Varroa mites falling off, where the highest average of dropping was 90.60% when fumigating neem leaves after 24 hours of control, followed by fumigation with rosemary leaves by 85.04% after 24 hours of treatment, followed by the treatment of thyme leaves after 48 hours of control, which amounted to 83.85%.While the fumigation treatment with olive tree leaves recorded the lowest dropping percentage 29.00% after 48 hours of the control process compared to the control treatment that was fumigated with burlap and canvas only, where the dropping percentage was 21.74% after 72 hours of treatment. The results showed that there were significant differences between the treatments of plant extracts and the time of control, as the highest dropping percentage reached 94.27% when treated with neem leaf extract after 48 hours of control and the lowest dropping percentage when treated with olive leaf extract, which amounted to 26.25% after 72 hours of control compared to treatment The control, which recorded the lowest dropping percentage of 17.58% after 72 hours of control. While there are no significant differences between the control methods and the natural plant materials used in the percentages of honey bee mortality in the laboratory. The treatment with thyme oil also achieved the highest rate of honey hive production of 14.66 kg / hive and 14.36 kg / hive when fumigating with thyme leaves, followed by treatment with eucalyptose and neem oils at a production rate of (14.30 and 14.13) kg / hive, respectively, and the lowest production rate recorded. Fumigation treatment with olive leaves at a rate of 7.30 kg/cell compared to the control treatment, which recorded the lowest production rate of 5.86 kg/cell.

Keyword: Varroa jacobsoni ، Apis mellifera ، Azadirachta indica ، Thymus vulgaris

1- Introduction

Varroa jacobsoni is one of the most dangerous pests that significantly degrades infected beehives (Hunt et al., 2016; Evans and Schwarz 2011, Figen et al. 2012). They feed on the hemolymph of adults and pupae of honeybees and may feed on the fatty bodies of honeybees, causing severe physiological disturbances including a decrease in weight, body fluids, protein and carbohydrate level (Maggi et al., 2009 and Ramsey et al., 2019).Varroa infection leads to some deformations in the bee body, a decrease in production and the death of the entire beehives in case of severe infection, which led to the diagnosis of the parasite as one of the factors that lead to significant losses in beehives all over the world (Staveley et al., 2014, Carreck and Neumann, 2010 and Currie et al., 2010).Varroa has spread in Iraq and was recorded as an epidemic pest in 1987 in the city of Dohuk in northern Iraq (Mahdi et al., 2020 and Ministry of Higher Education and Scientific Research, 1987).After that, it spread throughout the governorates of Iraq, and its spread had a devastating effect on bee colonies in Iraq. The collapse in hives reached 90% (Al-Hasnawi and Al-Sami`, 2020).Several attempts were made to use various compounds to control Varroa mites, including synthetic pesticides such as amitraz, coumaphos, fluvalinate and many other chemical pesticides (1995, Milani and Elzen et al., 1999) to the fact that the excessive use of these pesticides led to the emergence of many resistant strains of mites Varroa mites as pesticide residues were observed in honey bee products (Sabahi et al. 2017 and Tsuruda et al. 2012). The difficulty of controlling Varroa parasites also lies in the fact that it lives and shelters inside the sealed hexagonal eyes of the brood (Daly et al., 1988 and Hoppe et al., 1989).As a result, there is an urgent need to use Varroa control methods to maintain the health and products of honey bees (Brasesco et al. 2017).Alternative methods of control have also received great attention recently due to the negative effects of chemical pesticides on honey bees, human health, and the environment, Aljedani (2017) and therefore it is necessary to find alternative pesticides from natural plants (Singh 2014, Li et al. 2017). Therefore, attention has been drawn towards the control of using plant essential oils, which are among the secondary metabolites that the plant produces during the growth stage (Su et al. 2012 and Al-Jadaani, 2018).Natural essential oils are a highly desirable alternative to synthetic products (Bakar et al. 2018).Products of plant origin have shown a wide range of biological activities including toxicity, anti-nutritional effects in parasites, and growth-regulatory properties (Damiani et al., 2011).In recent years, some commercial plant extracts have been introduced to control Varroa mites, where the pesticide ApiLife var and thymol as the main component showed positive results in controlling Varroa mites without any pesticide residues in beeswax (Imdorf et al. 1995).It can also be successfully controlled by the use of Thymovar, a specialized commercial Varroa mite based on volatile oils (Bollhalder, 1998)) A number of natural plant materials were used in the current study, such as Neem (Azadirachta indica), Thymus vulgaris, Eucalyptus obliqua, Rosmarinus officinali, and Olea europaea.

2- Materials and Methods

2-1: Study location

The study was conducted in the bee project of the Plant Protection Department in the Basra Agriculture Directorate, located in Al-Haritha sub-district, with 60 Lancaster hives containing ten bee frames and brood were divided into five experimental units (treatments), each treatment included three hives (replicates) in addition to the control treatment (control).

2-2: Plant and natural materials used in the control

The plants (Neem, thyme, eucalyptose, rosemary and olive) were selected because they contain aromatic compounds that may be fatal or repellent to Varroa mites. As indicated in the following table:

Active Compounds	family	scientific name	Plants
It is a natural insecticide found in the seeds of the neem tree. It is yellow to brown in color, has a bitter taste, and has a garlic/sulfur odor. Neem oil is a mixture of ingredients. Azadirachtin is the most active ingredient to repel and kill pests and can be extracted from neem oil.	Meliaceae	Azadirachta indica	Neem
Contains phenolic compounds such as carvacrol and thymol in limited concentrations and gamma-terpenine and p-cymene	Lamiaceae	Thymus vulgaris	thyme
The eucalyptus tree contains tannins, terpenes and flavonoids, and the leaves of eucalyptus trees contain volatile oil ranging from (4-5%).	Myrtaceae	Eucalyptus obliqua	eucalyptus
Volatile oils 1-2% composed of purinol, camphene, camphor and styol. The flavonoids (epiggenin - diosmin). The diterpene, picrosulfin, and rosmyrisin.	Labiatae	Rosmarinus officinali	Rosemary
The oil contains a high percentage of unsaturated fats, vitamin E, k, polyphenols, chlorophyll, pheophytin, sterols, squalene, and compounds that give it its aroma and flavour.	Oleaceae	Olea europaea	Olive

2-3: Effect of some control methods on the percentage of Varroa mite dropping off

2-3-1: Effect of some vegetable oils on the percentage of Varroa mites dropping off

A number of plant essential oils were used in the control, such as Neem oil, Azadirachta indica, Thymus oil, Thymus vulgaris, Eucalyptus obliqua, Rosmarinus officinali, and Olive oil (Olea europaea) brought from the local market (from one of the local markets).White sheets of size (A3) were used, marked with numbers and symbols according to their hive, then painted, saturated and completely covered with oil separately and placed at the base of each hive. As for the control treatment, the white paper was coated with petroleum jelly only, without treatment with a specific oil.

2-3-2: Effect of fumigating the leaves of some aromatic plants on the percentage of Varroa mite dropping off

Five natural plant materials were used for control, which were brought from local markets, including the leaves of the neem tree Azadirachta indica and the leaves of the thyme plant Thymus vulgaris.Leaves of Eucalyptus obliqua, Leaves of Rosmarinus officinali, Leaves of Olea europaea A quantity of 250 gm of dry leaves for each plant material was taken individually and placed in a smoking bellows with pieces of burlap and burned to generate smoke and fumes from different materials. After lifting the inner and outer covers of the hive and by four pressures on the blower for each hive, the process was repeated three times in succession. Then the hive gate was closed for 5-10 minutes for the purpose of dropping off the varroa on the base of the hive and collecting it later and counting it. As for the control treatment, it was smoked with burlap only without adding any vegetable matter. And for the purpose of calculating the number of varroa mites falling daily, white sheets of A3 size were used, treated with Vaseline for the purpose of varroa adhesion, and they were replaced daily for three consecutive days.

3-3-3: Effect of leaf extracts of some aromatic plants on the average of Varroa mite dropping off

The leaves were collected, dried, crushed, and ground by a ceramic mortar and placed in plastic bags and marked and then extracted in the insect laboratory of the Plant Protection Department of the College of Agriculture and prepared by cold extraction method weighing (500 g) of the powder of each plant separately and placed in 1000 ml beakers Ethyl alcohol 70%. The nozzle of the flasks was closed tightly, it was shaken well and left for three days and then placed in the electric shaker for 24 hours, and after the shaking process was completed, the solution was filtered by a piece of boring cloth and then filtered again using filter paper without impurities. Then the solution was placed in an Evaporatore Rotary with Vacum at a temperature of 50-60 °C to get rid of the solvent. Then the extract was placed in sterile and marked glass bottles and kept until use (Harborne, 1973).To evaluate the effectiveness of plant extracts, the control was carried out on the hives infected with Varroa by spraying method. The base solution for each extract was prepared by adding 10 g of the extract in 100 ml of distilled water to obtain a concentration of 10%, and from it the concentration of 1.0 mg/ml was prepared using the equation: -

$C1 \times V1 = C2 \times V2$

whereas:-

C1 = base solution concentration

V1 = volume of the base solution

C2 = Desired dilution concentration

V2 = volume of dilution required

Then a sugar solution was prepared at a ratio of 2:1 (2 water: 1 sugar) used to make a concentration (1.0) and the hives were sprayed using a 2-liter volumetric hand sprayer, with three hives for each treatment in addition to the control treatment, which was sprayed with sugar solution only. The readings were taken before and after the control and the numbers of Varroa mites falling on white A3 paper were calculated after (24, 48 and 72) hours of treatment, then the relative effectiveness of each treatment was calculated through the following equation (Hajeej et al., 2004):

$$\text{Relative effectiveness \%} = \frac{\textit{Average number of Varroa shedding after 24 hours of control} - \textit{average natural shedding before control}}{\textit{Varroa shedding number after 24 hours of control}} \times 100$$

-4: Effect of some control methods on the percentage of adult honey bee mortality

The experiment was conducted in the entomology laboratory for postgraduate studies at the College of Agriculture - University of Basra, under laboratory conditions at a temperature of 30-33° C ± 2 and relative humidity of 55-60%.For this study, a wooden box representing the division of a hive measuring 22.5 x 23 cm was used, containing three frames of honeybee brood and adults, with three replications for each treatment in addition to the control treatment. Then the readings were taken after 24, 48 and 72 hours for each treatment separately, and the killing rate was recorded. Then, the Orell and Schneider equation was used to measure the effectiveness of the pesticide mentioned in Shaban and Al-Mallah (1993) as follows:

$$\text{effectiveness of the pesticide\%} = \frac{\text{Treatment death rate} - \text{death rate in control treatments}}{100 - \text{death rate in control treatments}} \times 100$$

-5: Calculation of the production average of bee honey kg/hive

The honey production rate of the hive was calculated by sorting the honey by a manual sorter during the month of November of the year 2020 and then weighing the honey for each treatment separately.

3-7: Statistical Analysis

The laboratory experiment was conducted in a completely randomized design, C.R.D, while the field experiments were conducted according to the completely randomized design, R.C.B.D, as factorial experiments and with three replications. Also, all means were compared according to the least significant difference (L.S.D) method, at a probabilistic level of 0.01 for laboratory experiments and 0.05 for field experiments (Al-Rawi and Khalaf Allah, 1980). The results were analyzed according to Gen Stat discovery edition 3.

3- Results and Discussion

3-1: Effect of some vegetable oils on the percentage of Varroa mites dropping off

The results of the statistical analysis in Table (1) indicated that there were significant differences in the average percentage of Varroa mites dropping off after treatment with aromatic vegetable oils, where the treatment with neem oil recorded the highest dropping off rate of 75.44%, followed by treatment with thyme, eucalyptose and

rosemary oil, with a dropping off rate of (65.51 and 53). 24 and 43.25%), respectively, while the lowest dropping off rate was when treated with olive oil, which recorded 28.18% compared to the control treatment, in which the dropping off rate was 17.8%.Also, significant differences were found for the time factor in the percentage of dropping off of Varroa mites, as the highest dropping off rate was 56.69% after 48 hours of treatment, and the lowest dropping off rate was 34.80 after 24 hours of treatment. The interaction between the treatments and the control time had a significant effect on the percentage of dropping off, as it reached the highest rate (85.31%) for the neem oil treatment after 48 hours of treatment and the lowest rate of dropping off(20.74%) for the olive oil treatment after 24 hours of the control compared to the control treatment, which amounted to 14.76 % after 24 hours of treatment with Vaseline only. The reason may be that neem oil contains an active substance known as azadirachtin, and the complex compound tetranortripernoids or liminoids is the main active component of it. Azadirachtin is a pest repellent, but it is not toxic to beneficial insects such as honeybees (Alam et al., 1990).It is one of the effective compounds as nutritional inhibitors, which are included in the group of triterpenoids extracted from neem trees, Azadiracta indica, as they are highly effective against a wide range of insect pests (Al-Harbi, 2007).The result agreed with that reported by Melathopoulos et al (2000) when they performed a laboratory control to evaluate neem oil and extract in the management of major pests on honey bees Apis mellifera L. The use of neem led to a decrease in the growth of parasites on honey bees and proved highly effective against two types of mites on laboratory infected bees. The average mortality was between (50-90%) of Varroa jacobsoni after 48 hours of control, and the mortality rate of bees was reduced to 10%, which indicates that neem oil has physical properties in the mechanism of action of the toxicity. Mahmood (2014) found that the use of vegetable oils such as (neem oil, garlic oil, clove oil, tobacco extract and olive oil) in controlling Varroa mites are among the effective factors Al-Hasani (2003) indicated that the laboratory control of Varroa parasite with neem and eucalyptus oil led to a decrease in the infection rate to 100%, while Datura extracts reduced the infection rate to 85%, while peppermint extract reduced the percentage to 33.4% after four hours of control. Shekh and Ghafoor (2017) showed that the highest number of Varroa shedding at a rate of 1.747 was for thyme oil, while the lowest was at a rate of 1,000 for peppermint oil at 100% concentration compared to the control treatment that recorded a dropping off rate of 1.087. Some vegetable oils gave impressive results in combating Varroa mites, such as thyme oil (thymol), peppermint oil (menthol) and thyme oil mixed with other oils. and others, 1999).Anise oil (Anethole) achieved a high relative effectiveness of 1.5 ml of oil/hive at 89.2%, while it gave a relative effectiveness of 84.1% at a rate of 4 ml of oil/jet per hive (Hajeej, 2009).

Table 1: Effect of some vegetable oils on the percentage of Varroa mites dropping off

oils	Percentage of dropping off after treatment/hour			Oil average
	24 hours later	48 hours later	72 hours later	
neem oil	62.78	85.31	78.23	75.44
thyme oil	55.20	73.42	67.92	65.51
eucalyptus oil	28.50	74.52	56.69	53.24
Rosemary oil	26.85	54.60	48.31	43.25
olive oil	20.74	35.23	28.57	28.18
control	14.76	17.07	21.67	17.83
time average	34.80	56.69	50.23	47.24
L.S.D 0.05	oils =3.23	Time=2.28	Interaction=5.59	

-2: Effect of fumigating the leaves of some aromatic plants on the percentage of Varroa mite dropping off

The results of the statistical analysis in Table 2 showed that there were significant differences in the effect of fumigation by leaves of some plants and the time of control and the interaction among them in the percentage of varroa mites dropping off and eucalyptose and rosemary with a dropping off rate of (79.01, 73.41 and 61.87%), respectively. Compared to the control treatment in which fumigation was used with burlap only, the average dropping off rate was 24.47%.As for the effect of the control time factor, it gave significant differences in the percentage of Varroa mites dropping off , with the highest rate reaching 64.08% after 24 hours of control, while the lowest dropping off rate was 53.34% after 72 hours of treatment. The interaction between the treatments and the time factor of control had a significant effect, where the highest average of dropping off reached 90.60% when fumigating neem leaves after 24 hours of control, followed by fumigation with rosemary leaves by 85.04% after 24 hours of treatment, followed by the treatment of thyme leaves after 48 hours of control, which amounted to 83.85%,While the fumigation treatment with olive tree leaves recorded the lowest dropping off rate of 29.00% after 48 hours of the control process compared to the control treatment that was fumigated with burlap and canvas only, where the dropping off rate was 21.74% after 72 hours of treatment. The reason may be due to the fact that the active substance evaporated from plant smoke is in its highest concentration immediately after smoking, which leads to reactions shown by bees during the fumigation process that increase the dropping of Varroa. Rajiter (1983) also indicated that the incidence of Varroa mites was reduced by 50-79% when beehives were smoked with tobacco leaves for the purpose of control. And the population density of varroa infested when using some natural smoking materials, including powder (pepper seeds, mint leaves, eucalyptus leaves, and fenugreek leaves) increased at a rate of (63.52, 62.80, 48.55, and 28.76) varroa/hive, respectively, compared to the control treatment, in which the dropping off rate was 14.43 Varroa/hive (Kamander and Fadl, 2011)Hajjij (2009) showed that the use of some smoking plant materials achieved high relative effectiveness in the fall of varroa mites. The seeds of the anise plant (10 g / hive), the dried leaves of rosemary (15 g / hive), and the roots of the eucalyptus plant (20 g / hive) recorded effectiveness of (81.4, 80, and 79.1%), respectively. Compared to the control treatment in which the dropping off rate was 8.8%.

Table 2: Effect of fumigating the leaves of some aromatic plants on the percentage of Varroa mite dropping off

Aromatic plant leaves	Percentage of dropping off after treatment/hour			fumigating average
	24 hours later	48 hours later	72 hours later	
neem leaves	90.60	85.83	81.40	85.94
thyme leaves	80.35	83.85	72.83	79.01
eucalyptus leaves	69.29	63.21	53.10	61.87
rosemary leaves	85.04	74.24	60.95	73.41
olive leaf	32.20	29.00	30.04	30.41
control	27.00	24.67	21.74	24.47
time average	64.08	60.13	53.34	59.19
L.S.D 0.05	oils =0.77	Time=0.55	Interaction=1.34	

Effect of leaf extracts of some aromatic plants on Varroa mites dropping off

The results of the statistical analysis in Table 3 showed that there were significant differences between treatments in the percentage of Varroa mites dropping off. The aqueous extract of neem recorded the highest rate of varroa mite drop of 89.17%, followed by the treatment of aqueous extract of thyme, eucalyptus, and rosemary, with a rate of Varroa mite drop of (85.89, 75.48, and 70.74%) respectively, compared to the control treatment that was sprayed with water. Distilled only, with an average drop rate of 18.96%.As for the effect of the control time factor, it gave significant differences in the percentage of Varroa mites drop, with the highest rate reaching 64.95% after 48 hours of treatment, while the lowest dropping average was 59.82% after 72 hours of treatment. The results of the statistical analysis of the interaction showed that there were significant differences between the treatments and the control time, as the highest dropping average reached 94.27% when treated with neem leaf extract after 48 hours of control and the lowest dropping average when treated with olive leaf extract, which amounted to 26.25% after 72 hours of control compared to the treatment The control, which recorded the lowest drop rate of 17.58% after 72 hours of control. This may be due to the fact that neem plant extracts are broad-spectrum insecticides, meaning that they are highly effective against a wide range of pests and are not intended to exterminate a specific insect. The active compounds in neem extract are similar in composition to insect hormones. When these extracts are sprayed directly on the pests, their bodies absorb the compounds in the neem as real hormones and respond to those hormones, which leads to the glandular system in the pest stopping releasing hormones and thus completely disrupting the vital processes in the pest's body (Pineda et al., 2009)The neem extract is similar in its effectiveness to chemical insecticides even in extreme concentrations, and its effectiveness was tested against 200 types of harmful pests, some of which have immunity to chemical pesticides. (Tawfiq, 1997). Refaei (2011) showed when testing the extracts of four compounds (Apiguard, thyme, camphor, and basil) that it is highly effective in controlling Varroa mites, and that Apiguard is the most effective substance, with its efficiency (81.3, 74.6, 71, and 67%), respectively compared to the control treatment, which amounted to 14.6%.And Mahmood (2014) showed that the combination of clove oil and tobacco extract, when used at a concentration of 5% to control Varroa mites on honeybees, gave a precipitation percentage of 96.48%, while the tobacco extract alone gave a dropping average of (85.68%) after 24 hours of treatment. Nibets et al, (2002) explained that the effect of neem extracts does not usually kill pests, but it radically affects their behavior and development, as it works to prevent them from the process of feeding, mating and metamorphose and that the compound Azadirachtin is one of the unpalatable compounds for insects, as they prefer death by starvation than feeding with this compound. The extracts of neem trees also contain salanin, which is a powerful pest repellent and is more effective than DEET (N,N-diethylm-toluamide) insect repellent (Bose et al., 2009).Faraj et al. (2021) found that the use of lavender extract by spraying method to control Varroa mites was significantly superior to oxalic acid, garlic extract, and ginger in reducing the incidence of Varroa mites.

Table 3: Effect of leaf extracts of some aromatic plants on Varroa mites dropping rate

extract	Percentage of dropping off after treatment/hour			extract average
	24 hours later	48 hours later	72 hours later	
neem extract	85.07	94.27	88.17	89.17

Thyme extract	84.44	87.98	85.23	85.89
eucalyptose extract	73.52	77.93	74.98	75.48
rosemary extract	68.50	77.02	66.69	70.74
olive extract	35.20	31.09	26.25	30.85
the control	17.87	21.42	17.58	18.96
time rate	60.77	64.95	59.82	61.84
L.S.D 0.05	extract =1.32	Time=0.93	Interaction=2.28	

3-4: Effect of control methods on the percentage of adult honey bee mortality

The results of the statistical analysis in Table 4 showed that there were no significant differences between the control methods and the natural plant materials used in the percentages of honey bee mortality in the laboratory. The rates of mortality percentages of adult honey bees ranged between (26.2, 16.7, 21.7, 17.0 and 18.9%) for each of neem, thyme, eucalyptose, rosemary and olive, respectively, compared to the rate of the control treatment, which amounted to 17.6%.

Table 4: Effect of control methods on the percentage of adult honey bee mortality

Materials	Percentage of honey bee mortality			Materials average
	Oil	fumigating	extracts	
neem	26.8	26.7	25.2	26.2
thyme	18.3	15.2	16.7	16.7
eucalyptose	18.3	21.7	25.0	21.7
Rosemary	16.0	15.0	20.0	17.0
olive	18.3	20.0	18.3	18.9
the control	16.7	18.3	16.7	17.2
control rate	19.1	19.5	20.3	19.6
L.S.D 0.05	Materials=9.67	control =6.84	Interaction =2.28	

3-5: Effect of control methods on the rate of bee honey production kg/hive

The results of the statistical analysis in Table 5 showed significant differences between the control method and the natural plant materials used in the total production rate of the hive, where the treatment with thyme plant achieved the highest production rate of 14.12 kg/hive, followed by the treatment of eucalyptus, neem and rosemary with a production rate of (12.18 and 11.71). and 9.16) kg/hive, respectively, while the olive plant treatment gave the lowest production rate of 8.06 kg/hive compared to the control treatment in which the production rate was 6.50 kg/hive. The control method also achieved significant differences in the total production of the hive, as the highest rate was 11.30 kg / hive when controlling with volatile vegetable oils, while the lowest rate was 9.68 kg / hive when using the aqueous extract method in the control. The results of the bi-interaction showed that there were significant differences in the rate of production, so the treatment with thyme oil achieved the highest rate of hive production, which amounted to 14.66 kg/hive and 14.36 kg/hive when

fumigated with thyme leaves, followed by treatment with oils of both eucalyptus and neem at a production rate of (14.30 and 14.13) kg/ hive, respectively, and the lowest production rate recorded by fumigation with olive leaves at a rate of 7.30 kg/hive compared to the control treatment, which recorded the lowest production rate of 5.86 kg/hive.The reason may be due to the effectiveness of the control, the decrease in the number of Varroa and the increase in the density of bees, and thus the increase in the strength of the colony. The results agreed with Arechavaleta-Velasco and Guzmán-Novoa (2001) that the control hives gave the highest rate in terms of honey production compared to the non-control hives. Subbotin and Orlva (1976) and Shawer (1987) confirmed the existence of a positive relationship between honey production and colony strength, and that the strength of the colony has a significant impact on the collection and consumption of pollen, and the supply of honey bees with pollen before winter leads to an increase in bee density in the spring, Which results in an improvement and increase in honey production and overcoming of bad weather conditions in winter (Jevtic et al., 2005 and Mattila and Otis 2006). Al-Hasnawi (2019) mentioned that the rate of honey production in Basra province was 3 kg / hive, as the city district exceeded the production rate by 5 kg / hive, while the production between districts ranged from 2.5-3 kg / hive. Faraj and others (2021) showed that the use of three substances represented by lavender, garlic and ginger in controlling Varroa parasite in three different ways, in the form of extracts, smoking and dusting with their powders, increased honey production by an average of 170,000, 53,667 and 28,333 ng2 by extracting and smoking it amounted to (91.667, 96.667 and 141.667). Ang 2, and dusting (38.333, 10,000 and 131,667) inches 2 for each of lavender, garlic and ginger, respectively, compared to the control, which yielded 1.667 inches 2, as the control increased bee density, increased sealed and unsealed brood and pollen area, which led to an increase in the area of production Honey.

Table 5: Effect of control methods on the rate of bee honey production kg/hive

Materials	Production average of bee honey kg/hive			Materials average
	Oil	fumigating	extracts	
neem	14.13	10.10	10.90	11.71
thyme	14.66	14.36	13.33	14.12
eucalyptose	14.30	11.66	10.60	12.18
Rosemary	9.33	8.83	9.33	9.16
olive	8.83	7.30	8.06	8.06
the control	6.55	7.08	5.86	6.50
control rate	11.30	9.89	9.68	10.29
L.S.D 0.05	Materials=0.38	control =0.53	Interaction =**0.92**	

Conclusions

The results of the study showed the superiority of neem oil, smoking and extract of neem leaves in recording the highest rate of varroa mites dropping (75.44, 85.94 and 89.17)%, respectively, within 48 hours of treatment, and that the lowest rate was recorded by olive oil and smoking of mites in varroa mites dropping and reached (28.18 and 30.41). and 30.85%) respectively after 72 hours of treatment, and the highest rate of honey production achieved by thyme oil was 14.66 kg/hive and 14.36 kg/hive when fumigated with thyme leaves.

Acknowledgments

The authors are very much grateful to the Department of Plant Protection - College of Agriculture - University of Basrah for support this study.

6. References

Tawfiq, Muhammed Fouad (1970). General entomology. Faculty of Agriculture - Cairo University.3913 Knowledge Press in Egypt 483 pages.

Al-Jadaani, Dalal Mosleh (2018). Efficacy of some plant essential oils to control Varroa destructor on honey bees Apis mellifera L. Journal of King Abdulaziz University-Science. 30(2): 57-74.

Hajjij, Noureddine Zahir, Al-Baraqi, Ali Khaled. (2004). Varroa Jacobsoni Oud Anti-Varroa bee smoke for natural substances. The Third International Conference of the Arab Beekeepers Union - Tanta - Arab Republic of Egypt 28-31.

Hajjij, Noureddine Zahir, Al-Baraqi, Ali, Al-Abed, Tammam. (2009). The effectiveness of using oxalic acid by some methods in controlling the parasite Varroa destructor (Varroa Jacobsoni Oud) and its effect on the bee colony, Damascus University Journal of Agricultural Sciences, Volume 25, Issue 2.

Al-Harbi, Mona Ghoneim Ahmed (2007). Effect of Azadirachta indica neem extracts on the adult stage of Rhizopertha dominica (F.) and on liver and kidney tissues in albino rats. Master Thesis. College of Science - Department of Biology - King Abdulaziz University.

Al-Hasnawi Montaser Sabah Mahdi (2019). Spatial analysis of the spread of Varroa parasite and its effect on honey bees in Iraq. Ph.D. University of Kufa. college of Literature. Geographical department. 288 pages.

Al-Hasnawi, Montaser Sabah, Al-Sami', Mahmoud Badr. (2020). The effect of temperature on the spread of Varroa parasite and methods of controlling it in Iraq. Journal of Geographical and Cartographic Research Center. 17(30): 153-185.

Al-Hassani, Aref Muhammad Ahmad Ali (2003). An ecological study of the Varroa jacobsoni nipple parasitizing on honey bees and evaluation of the efficacy of a number of methods to combat it. Master's thesis, College of Agriculture, University of Aden, Honey Bee Magazine, Arab Beekeepers Union, No. 3: 61 pages.

The narrator, Khashi' Mahmoud; Abdul Aziz Muhammad Khalaf Allah (1980). Design and analysis of agricultural experiments - College of Agriculture and Forestry - University of Mosul, Dar al-Kutub for printing and publishing. 488 pages.

Shaaban, Awad; Mellah, Nizar Mustafa (1993). pesticides. University of Al Mosul. Ministry of Higher Education and Scientific Research. 520 pages.

Kamander, by night, Muhammad Saeed; Fadl, Salem Muhammad. (2011). Control of Varroa destructor on honey bees with smoke of some plant extracts. Yemeni Journal of Research and Studies. (24): 129-136.

Ministry of Higher Education and Scientific Research (1987). Mosul University, College of Agriculture and Forestry, Report, No. 9-3-6397.

Alam, M. M., Siddiqui, M. B., & Husain, W. (1990). Treatment of diabetes through herbal drugs in rural India.

Fitoterapia, 61(3): 240-242.

Aljedani, D. M. (2017). Detection of toxicity and effects of some insecticides to local honey bee race (Apis mellifera jemenatica). Journal of American Science, 13(3): 19-31.

Arechavaleta-Velasco, M. E., & Guzmán-Novoa, E. (2001). Relative effect of four characteristics that restrain the population growth of the mite Varroa destructor in honey bee (Apis mellifera) colonies. Apidologie, 32(2): 157-174.

Bakar, M. A., Aqueel, M. A., Raza, A. B. M., Arshad, M., Mahmood, R., & Qadir, Z. A. (2018). Comparative efficacy of five commercial synthetic acaricides against Varroa destructor (Anderson and Trueman) in Apis mellifera L. colonies. Pakistan Journal of Zoology, 50(3): 857-861.

Bollhalder F (1998). Thymovar for Varroa control. Schweiz Bienen-Zeitung 121:148–151.

Bose, A., Chakraborty, K., Sarkar, K., Goswami, S., Haque, E., Chakraborty, T., ... & Baral, R. (2009). Neem leaf glycoprotein directs T-bet–associated type 1 immune commitment. Human immunology, 70(1): 6-15.

Brasesco, C., Gende, L., Negri, P., Szawarski, N., Iglesias, A., Eguaras, M., ... & Maggi, M. (2017). Assessing in vitro acaricidal effect and joint action of a binary mixture between essential oil compounds (thymol, phellandrene, eucalyptol, cinnamaldehyde, myrcene, carvacrol) over ectoparasitic mite Varroa destructor (Acari: Varroidae). Journal of apicultural science, 61(2): 203-215.

Carreck, N., & Neumann, P. (2010). Honey bee colony losses. J Apic Res, 49(1): 1-6.

Daly, H. V., De Jong, D., & Stone, N. D. (1988). Effect of parasitism by Varroa jacobsoni on morphometrics of Africanized worker honeybees. Journal of apicultural research, 27(2): 126-130.

Damiani, N., Gende, L. B., Maggi, M. D., Palacios, S., Marcangeli, J. A., & Eguaras, M. J. (2011). Repellent and acaricidal effects of botanical extracts on Varroa destructor. Parasitology research, 108(1): 79-86.

Elzen, P. J., Eischen, F. A., Baxter, J. R., Elzen, G. W., & Wilson, W. T. (1999). Detection of resistance in US Varroa jacobsoni Oud.(Mesostigmata: Varroidae) to the acaricide fluvalinate. Apidologie, 30(1): 13-17.

Figen K., girisgin, A. O. aydin, L. (2012). Varroacidal efficacies of essential oils extracted from Lavandula officinalis, Foeniculum vulgare, and Laurus nobilis in naturally infested honeybee (Apis mellifera L.) colonies. Turk. J. Vet. Anim. Sci. 36, 554–559.

Harborne, J. B. (1973). Phenolic compounds. In Phytochemical methods (pp. 33-88). Springer, Dordrecht.

Hoppe, H., W. Pritter and W.C. Stephen Elizabeth, (1989). The control of parasitic mites: Varroa jacobsoni, Acarpis woodi and Tropilaelps clareaea with formic acid. Am. Bee j., 129: 739-742.

Hunt, G., Given, J. K., Tsuruda, J. M., & Andino, G. K. (2016). Breeding mite-biting bees to control Varroa. Bee Culture, 8, 41-47.

Imdorf, A., Bogdanov, S., Kilchenmann, V., & Maquelin, C. (1995). Apilife VAR: a new varroacide with thymol as the main ingredient. Bee World, 76(2): 77-83.

Li, L., Lin, Z. G., Wang, S., Su, X. L., Gong, H. R., Li, H. L., ... & Zheng, H. Q. (2017). The effects of clove oil on the enzyme activity of Varroa destructor Anderson and Trueman (Arachnida: Acari: Varroidae). Saudi journal of biological sciences, 24(5): 996-1000.

Maggi, M. D., Sardella, N. H., Ruffinengo, S. R., & Eguaras, M. J. (2009). Morphotypes of Varroa destructor collected in Apis mellifera colonies from different geographic locations of Argentina. Parasitology research, 105(6): 1629-1636.

Mahdi, M. S., Ali, M. B., & AL-abedy, A. N. (2020). Genetic variability of the Mite Varroa destructor Isolated from Honey Bees in Iraq and Some Middle Eastern Countries. Indian Journal of Forensic Medicine & Toxicology, 14(1): 1-7.

Mahmood, R., Asad, S., Raja, S., Ul Moshin, A., Wagchoure, E.S., Sarwar, G., Islam, N. and Ahmad, W. (2014). Control of Varroa destructor (Acari: Varroidae) in Apis mellifera (Hymenoptera: Apidae) by using plant oils and extract. Pakistan J. Zool. 46(3):609–615.

Mahmood, R., Wagchoure, E. S., ul Mohsin, A., Raja, S., & Sarwar, G. (2012). Control of ectoparasitic mites in honeybee (Apis mellifera L.) colonies by using thymol and oxalic acid. Pak. J. Zool, 44(4): 985-989.

Melathopoulos, A. P., Winston, M. L., Whittington, R., Smith, T., Lindberg, C., Mukai, A., & Moore, M. (2000). Comparative laboratory toxicity of neem pesticides to honey bees (Hymenoptera: Apidae), their mite parasites Varroa jacobsoni (Acari: Varroidae) and Acarapis woodi (Acari: Tarsonemidae), and brood pathogens Paenibacillus larvae and Ascophaera apis. Journal of economic entomology, 93(2): 199-209.

Milani, N. (1995). The resistance of Varroa jacobsoni Oud to pyrethroids: a laboratory assay. Apidologie, 26(5): 415-429.

Nibets, C. P.W, AS Costa and A.L. Lourencae (2002). Effect of som crude and aAzadaracctin enriched neem (Azairachta indica) seed kernel extracts on larvae of Aedes aegypti Entomol Exp Appl,35:11-16.

Pineda, S., Martínez, A. M., Figueroa, J. I., Schneider, M. I., Del Estal, P., Viñuela, E., ... & Budia, F. (2009). Influence of azadirachtin and methoxyfenozide on life parameters of Spodoptera littoralis (Lepidoptera: Noctuidae). Journal of Economic Entomology, 102(4): 1490-1496.

Rajiter, A. (1983). Preliminary results on treatment of varroa infected honey bee colonies with tobacco smoke. Bee World, 64(3): 63-65.

Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J. D., Cohen, A., Lim, D., Joklik J., Cicero J., Ellis J., van Engelsdorp D. & Hawthorne, D. (2019). Varroa destructor feeds primarily on honey bee fat body tissue and not hemolymph. Proceedings of the National Academy of Sciences, 116(5): 1792-1801.

Refaei, G. S. (2011). Evaluation of some natural substances against Varroa destructor infesting honeybee, Apis mellifera in Egypt. Egyptian Journal of Agricultural Research, 89(1): 169-175.

Sabahi, Q., Gashout, H., Kelly, P. G., & Guzman-Novoa, E. (2017). Continuous release of oregano oil effectively and safely controls Varroa destructor infestations in honey bee colonies in a northern climate. Experimental and Applied Acarology, 72(3): 263-275.

Sakofski, F., Koeniger, N., & Fuchs, S. (1990). Seasonality of honey bee colony invasion by Varroa jacobsoni Oud. Apidologie, 21(6): 547-550.

Shekh, M. S., Ghafoor, T. H. (2017). EVALUATION OF SOME PLANT OILS FOR CONTROLOF VARROA MITE (Vharroa jacobsoni Oudemans IN HONEY BEE (Apis mellifera Linnaeus, Hymenoptera Apidae) COLONIES. Euphrates Journal of Agriculture Science, 9(3): 62-78.

Singh, D. (2014). Management of Mite Pests in Honeybee Colonies Through Botanicals. In Advances in Plant Biopesticides (pp. 271-277). Springer, New Delhi.

Staveley, J. P., Law, S. A., Fairbrother, A., & Menzie, C. A. (2014). A causal analysis of observed declines in managed honey bees (Apis mellifera). Human and Ecological Risk Assessment: An International Journal, 20(2): 566-591.

Su, X., Zheng, H., Fei, Z., & Hu, F. (2012). Effectiveness of herbal essential oils as fumigants to control Varroa destructor in laboratory assays. Chinese Journal of Applied Entomology, 49(5): 1189-1195.

Tsuruda, J. M., Harris, J. W., Bourgeois, L., Danka, R. G., & Hunt, G. J. (2012). High-resolution linkage analyses to identify genes that influence Varroa sensitive hygiene behavior in honey bees. PloS one, 7(11): e48276.

Faraj, T. H. S., Rashed, R. J., Lazim, Z. S., & Atta, D. M. (2021). Study the effect of some plants extracts to control Varroa destructor Anderson and Trueman mite (Acari: Varroidae), and activity of honey bees (Apis mellifera L.),(Hymenoptera: Apidae). Euphrates Journal of Agriculture Science, 13(3): 60-69.

Subbotin, L.N and Orlva, S. F.(1976). Correlation between the most important chant characteristics of mergral strain of grey mountain Caucasian honeybee. Apicultural. 29(4): 245.

Shawer,M.B.(1987). Major pollen sources in kafer-EL-sheikh, Egypt, and the effect of pollen Supply on brood area and honey yield. J.Apic.Res.,126:43-46.

Jevtic, G., Mladenovic, M. and Nedic, N.(2005). the influence of the quantity of honeybees and honey reserves on wintering of honeybee colonies. Biotechnology in animal husbandry.,21(5-6):315-321.

Mattila, H.R. and Otis, G.W.(2006). Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies. J.Econ. Entomol. 99(3): 604-613.