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)

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Abstract

The study was carried out from April to late August 2021 at Nawrrah Bab apiary located in Sharbazher- Sulaimani. The highest average number of frames covered by adult castes (honeybee density) treated with lavender spraying were (6.667, 6.667 and 6.000) frame after (12, 24, 36) days of the treatment respectively. The highest rates of sealed and unsealed workers brood areas were (446.667, 410.000, 194.667) inch². and (438.333, 447.000, 225.333) inch² for sealed and unsealed after (12, 24, 36) days successively, Maximum pollen grain areas were (63.333, 62.000, 76.667) inch². when treated with Lavender Spray after twelve, twenty four and thirty six days alternatively, while the highest rates of sealed honey areas were (170.000, 240.000 and 352.667) inch² when treated with lavender Spray after twelve, twenty four and thirty six days respectively. Ginger smoking obtained the highest taste value which is superior significantly to the others except garlic soaking. No significant differences were found for odor among the treatments. Lavender spray gave the maximum value for color which is different significantly from the others except that of garlic soaking, ginger spray, and control. Lavender spray resulted the highest value for consistency which is superior significantly to the others. Also, lavender spray recorded the highest value for mite mortality which is superior to both garlic spray and control.

The objective of this study was to investigate the effect of medicinal plants as alternative chemical pesticides in controlling *Varroa* Mites and increasing honeybee activity.

Introduction

The western honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) is considered as the most vital pollinator of wide diverse of agricultural crops worldwide (Kingston *et al*., 2018). Honeybee products (wax, honey, royal jelly propolis,etc.) have nutritional and medicinal importance (Al-Kenawy *et al*., 2021 and Rossi and Marrazzo, 2021). Honeybee decline in the world refers to some factors; viruses (Allen and Ball, 1996), herbicides (Iwasa *et al*., 2004 and Johnson *et al*.,2010) , and Varroa (Le Conte *et al*., 2010 and Fransic, 2013).

The mite, *Varroa destructor* Anderson and Trueman is the most damaging parasite of the honeybee facing beekeepers throughout the world nowadays (Anderson and Trueman, 2000). A great amount of honeybee colony losses in many countries because of Varroa infestation was recorded (Le Conte *et al*., 2010 and Eliash and Mikheyev, 2020). Kanga and James, 2002 found the decrease of up to 25% of adult bee weights, reducing workers and drone life in addition to wings deformation via feeding on the haemolymph of adult bees and brood.

Control of Varroa is through the use of synthetic acaricides especially pyrethroids. However, in many cases, the continuous use of these chemicals throughout years may cause

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some problems like the contamination of both wax and honey when applied within the hives (Wallner, 1999 and Jamal *et al*., 2020). Therefore, the recent approach to control this mite is through the use of relatively cheap and safe methods such as mechanical, biological managements, and essential oils (Allam *et al*., 2021, Han *et al*., 2021 and Mesbah *et al*., 2021). These methods could be used alone or in combinations to amplify their effectiveness (Milani, 1995).

Acaricide properties of some botanical extracts to control varroa have been tested at different locations in the world. Ariana et al. (2002) used 2% water extraction of lavender and recorded more than 95% varroa mortality. Other researches showed up to 77% mortality through the use of Garlic extract (Goswami and Khan 2013, Qayoum *et al*. 2013 and Goswami *et al*. 2014). Also, higher potency of garlic extract was observed by Al-Kenawey *et al*. (2021).

In order to decrease harmful effects of chemical usage on the environment and human health, environmentally friendly and healthy products are widely encouraged worldwide. Thus, the current study aims to use three botanical extracts (Lavender, Garlic and Ginger) against Varroa mite in beehives.

Materials and Methods

The extractions were carried out in the Laboratory of Horticulture department, College of Agricultural Engineering Sciences/ University of Sulaimani. Three different plant leaf powders (Garlic, Ginger and Lavender) were used for extraction for the purpose of spray and soaking methods. 60g from each plant powder were placed in a tightly covered, a volume liter conical flask containing 600 ml of distilled water which kept in water bath at 38˚C for two hours with continuous shaking each 20 minutes. The extractions were then cooled out and kept in dark for 24 hours before filtration through transparent fabrics, the filtrates then placed in containers which wrapped with black poly ethylene bags and transferred quickly to the field. For oxalic acid preparation: a mixture of 300g sugar and 300ml of lukewarm water was used, after cooling, 18g oxalic acid was added to the mixture and sprayed on the bee frames. For soaking, a piece of cotton was saturated with plant extracts and placed on the frames. For fumigation: Ten grams from each plant powder were burned in the smoker and applied directly to each replicate. Thick white paper sheet soaked with oil was placed at the bottom of each hive for the purpose of varroa collection.

Each tested colony treated with the extractions, oxalic acid and control at seven - day intervals for a month period. Mite mortality percentage was taken after a day of the treatment application according to the following formula:

$$Percent\ mite\ mortality(\%) = \frac{Mite\ mortality\ in\ treatment}{Mite\ mortality\ in\ treatment + Mite\ mortality\ in\ control} * 100$$ (Pawar, 2008).

At the end of experiment (after a month) the following parameters were taken thrice at 12-day intervals: density of adult worker bees/frame, sealed and unsealed brood (in^2), pollen grain (in^2), and area of sealed honey (in^2).

Sensory evaluation

In order to evaluate sensory evaluation of honey (taste, odor, color and consistency) treated with three types of plant extracts (Lavender, Ginger and Garlic) in addition to oxalic acid and control in the laboratory, the honey was introduced to 33 persons (female and males) familiar with honey bees. The scores given were (40, 20, 20 and 20) % for taste, odor, color and consistency `respectively.

Statistical Analysis*

The results were analyzed statistically using CRD design with triple replicates and performed using XLSTA program (2017) m, Duncan's multiple range Test was used to

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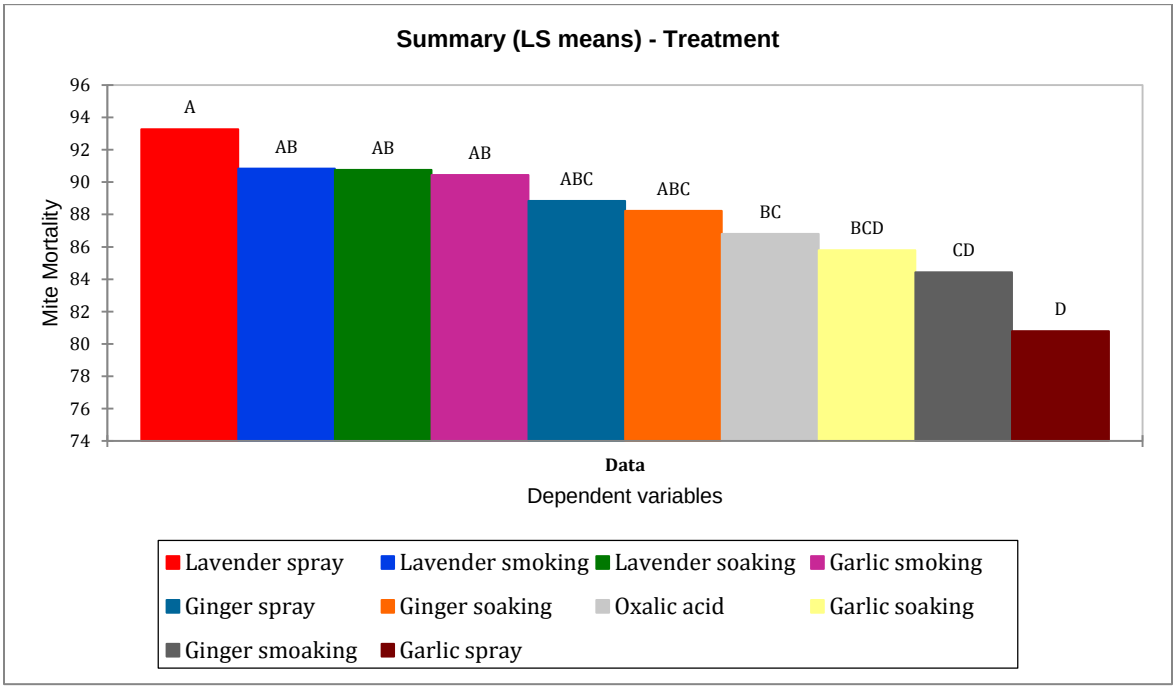
determine the differences between means at P=0.05 and determine the correlation coefficient(r) .

Result and Discussions

Mite Mortality

Figure 1 shows that lavender spray was superior significantly to oxalic acid, garlic soaking, ginger smoking, and garlic spray.



The results disagree with (Taha et al.,2020) who stated that the garlic oil was superior in reducing the number of varroa mites. In addition, they are incongruent with (Ariana et al.,2002) who showed that the lavender is not different significantly with the control.

The result agrees with Ariana et al., 2002 who mentioned that 2% lavender extract were most effective in controlling mite.

Table 1 shows that lavender spray gave the highest numbers (6.667, 446.667 in², 438.333 inch², 63.333 in², and 170.000 inch²) for density of adult workers/frame, sealed brood area, unsealed brood area, pollen grain area, and area of sealed honey respectively which are superior significantly to most other treatments.

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Table (1) Effect of plant extracts on honey bee activity (12 days after the study)

Treatments	density of adult workers/ frame	area of sealed brood (in^2)	area of unsealed brood (in^2(	area of pollen grain (in^2)	area of sealed honey (in^2)
Control	5.000 b	30.000 c	73.333 d	13.333 b	1.667 c
Garlic Spray	6.000 ab	270.000 b	333.333 ab	44.000 ab	53.667 a-c
Garlic Soaking	5.000 b	88.333 c	190.000 b-d	37.000 ab	10.000 c
Garlic Smoking	5.667 ab	69.333 c	285.000 a-c	36.000 ab	96.667 a-c
Lavender Spray	6.667 a	446.667 a	438.333 a	63.333 a	170.000 a
Lavender Soaking	6.000 ab	100.000 c	200.000 b-d	24.333 ab	38.333 bc
Lavender Smoking	5.333 ab	44.000 c	90.000 cd	25.000 ab	91.667 a-c
Ginger Spray	5.667 ab	333.333 ab	200.000 b-d	16.333 b	28.333 bc
Ginger Soaking	4.667 b	76.667 c	95.000 cd	21.333 b	131.667 a-c
Ginger Smoking	6.000 ab	78.333 c	275.000 a-d	15.000 b	141.667 ab
Oxalic acid	4.667 b	61.667 c	116.000 cd	28.333 ab	86.667 a-c

Means with the same letter are not different significantly at 0.05 level according to Duncan's multiple range test.

Table 2 indicates that the lavender spray, lavender smoking, and ginger smoking gave the highest density of adult workers (6.667) which is superior significantly to both control and oxalic acid, while no significant differences are noticed among the other treatments. Also, Lavender spray gave the highest number (410.000 $inch^2$) for sealed broods which is superior significantly to most other treatments. Again, lavender spray gave the highest number (447.000 $inch^2$) unsealed broods which is superior significantly to some other treatments. While, no significant differences were noticed for pollen grain areas with regard to all the treatments. On the other hand, Lavender smoking gave the highest value (240.000 $inch^2$) of honey yield which is not different significantly from lavender spray, Ginger smoking, Ginger soaking, and Garlic smoking, whereas it is superior to the rest of the treatments.

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Table (2) Effect of plant extracts on honey bee activity (24 days after the study)

Treatments	density of adult workers/frame	area of sealed broods (in²)	area of unsealed broods (in²)	area of pollen grains (in²)	area of sealed honey (in²)
Control	5.333b	80.667 c	91.667 de	30.667 a	20.333 d
Garlic Spray	6.333 ab	297.000 ab	353.333 ab	61.667 a	71.667 b-d
Garlic Soaking	6.000 ab	108.333 bc	193.333 b-e	50.333 a	61.667 b-d
Garlic Smoking	6.000 ab	210.000 bc	268.667 a-d	62.000 a	141.667a-d
Lavender Spray	6.667 a	410.000 a	447.000 a	85.000 a	230.000 a
Lavender Soaking	6.000 ab	108.333 bc	226.667 b-e	35.667 a	156.667 a-c
Lavender Smoking	6.667 a	75.667 c	139.333 c-e	54.667 a	240.000 a
Ginger Spray	6.000 ab	263.333 a-c	325.333 a-c	37.667 a	32.667 cd
Ginger Soaking	6.333 ab	72.333 c	178.667 b-e	36.000 a	173.333 ab
Ginger Smoking	6.667 a	131.667 bc	216.333 b-e	42.333 a	178.667 ab
Oxalic acid	5.000 b	87.667 c	71.667 e	44.667 a	99.667 b-d

Means with the same letter are not different significantly at 0.05 level according to Duncan's multiple range test.

Table 3 illustrates that both garlic soaking and lavender spray gave the highest number of density of adult workers/frame (6.000) which is superior significantly to both control and oxalic acid. With, no significant differences compared with the others. While, lavender spray, Ginger spray, and garlic spray gave highest numbers (194.667, 180.000, and 164.000) inch² of sealed brood successively which are superior significantly to all other treatments. Lavender spray gave the highest number of unsealed brood areas (225.333 inch²) which is superior significantly to control, lavender smoking, ginger soaking, and oxalic acid. Lavender spray recorded the highest number of pollen grain areas (76.667 inch²) which prevails significantly the most other treatments. Lavender spray dominates significantly the most other treatments with regard to honey sealed area.

Table (3) Effect of plant extracts on honey bee activity (36 days after the study)

Treatments	density of adult workers/ frame	area of sealed brood (in²)	area of un sealed brood (in²)	area of pollen grain (in²)	area of sealed honey (in²)
Control	4.667 b	24.000 b	41.333 c	27.667 b	25.667 d
Garlic Spray	5.333 ab	164.000 a	172.000 a-c	55.333 ab	116.667 b-d
Garlic Soaking	6.000 a	67.333 b	153.667 a-c	49.000 ab	52.667 cd
Garlic Smoking	5.667 ab	50.333 b	182.000 ab	55.333 ab	217.000 a-c
Lavender Spray	6.000 a	194.667 a	225.333 a	76.667 a	352.667 a
Lavender Soaking	5.667 ab	62.667 b	151.333 a-c	33.000 b	97.000 cd
Lavender Smoking	5.333 ab	40.667 b	62.333 bc	39.333 ab	141.000 b-d
Ginger Spray	5.333 ab	180.000 a	136.000 a-c	25.000 b	106.667 b-d
Ginger Soaking	5.000 ab	47.333 b	55.000 bc	34.000 b	210.333 a-c
Ginger Smoking	5.333 ab	53.667 b	147.667 a-c	31.667 b	281.667 ab
Oxalic acid	4.667 b	50.333 b	77.000 bc	27.667 b	143.000 b-d

Means with the same letter are not different significantly at 0.05 level according to Duncan's multiple range test.

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The results agree with both Subbotin and Orlva (1976) and Shawer (1978) who showed positive relationships between honey production and colony strength. Also, they agree with Jevtic., *et al* (2005) who recorded that the colony strength has a high influence on the gathering and consumption of the pollen grain, again the results agreed with Matilla and Otis (2006) who found that supplying honey bees with pollen diet before wintering causes the increase of large spring population but in a long run, honey yield improvement may result when bad weather condition reduces the spring foraging.

Figure2 indicates that ginger smoking recorded the highest value for taste which is not different significantly from the other treatments except garlic soaking.

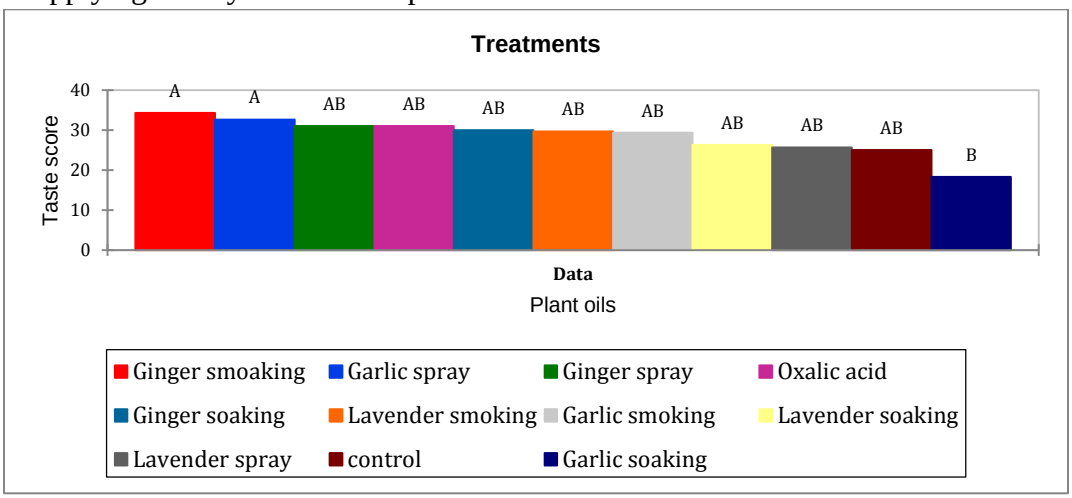

Figure (2): Taste scores of honey treated with botanical oils of lavender, ginger and garlic in addition to oxalic acid and control.

Figure 3 shows no significant differences among the treatments with regard to odor.

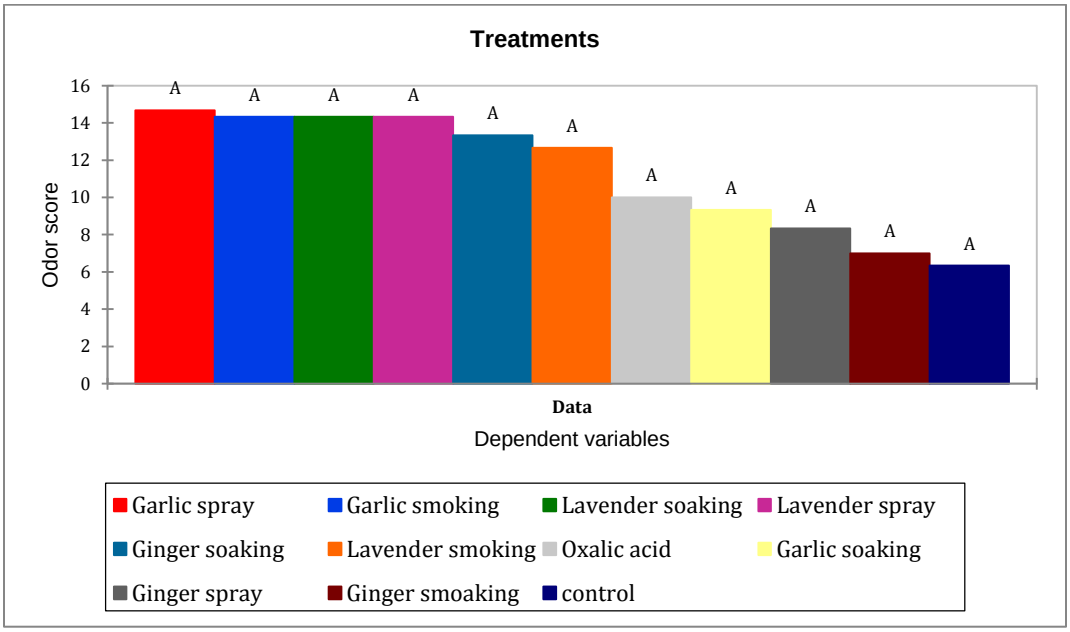

Figure (3): Odor sores of honey treated with three types of oils in addition to oxalic acid and control.

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Figure 4 illustrates that lavender spray gave the maximum value for color scores which is not different significantly from the other treatments except garlic soaking, ginger spray and control.

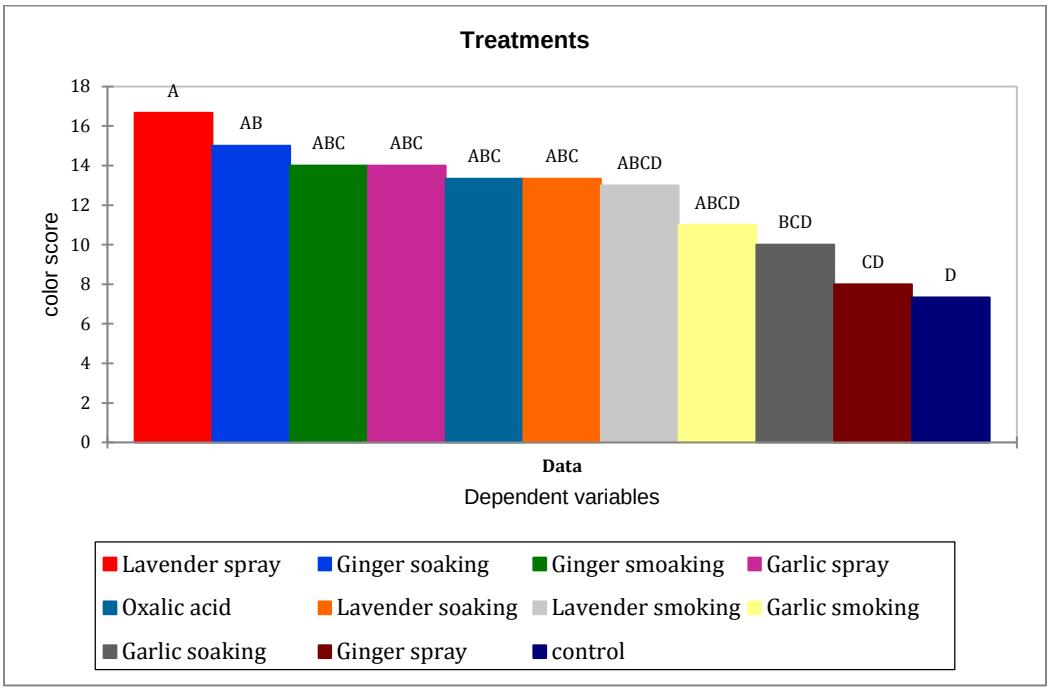

Figure (4): Color sores of honey treated with three types of oils in addition to oxalic acid and control.

Figure 5 exhibits that lavender spray recorded the highest value for consistency which is superior significantly to the other treatments.

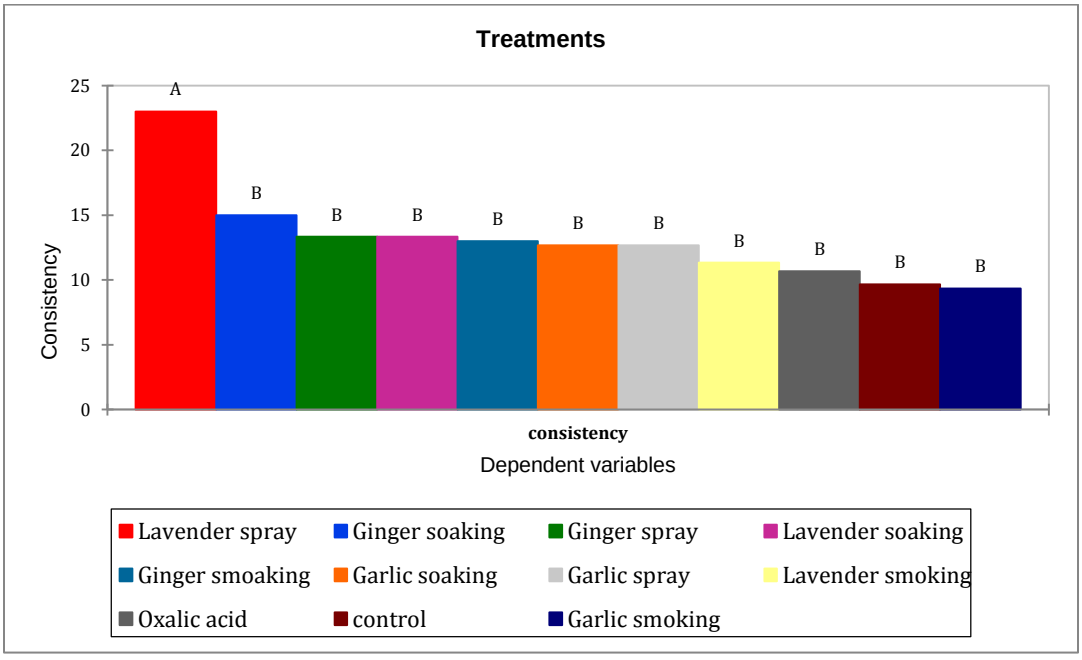

Figure (5) Consistency scores of honey treated with three types of oils in addition to oxalic acid and control

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The sensory evaluation; taste, color, odor and consistency of the collected honey from the studied hives revealed different results on the quality of the honey. The odor test of all honey samples has shown no change while the rest of the test have shown significant effect on the quality of the honey. These results declare that the components of bee feeding or using different extracts for controlling varroa purposes may affect the quality of the honey (Chukwu et al., 2012) and (Kaakeh and Gadelhak, 2005).

In conclusion, three different extracts lavender, garlic and ginger was applied in three different methods to control the varroa mite. The lavender extract applied in spray method is effective in causing mite mortality. In addition, this application increases bee density, high sealed brood, unsealed brood, honey and pollen grain area. This outcome should be followed thereafter by all beekeepers for achieving better control, healthy apiary and organic products.

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