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Biological activity of certain natural products against *Varroa destructor* (Acari:Varroidae) and their selectivity against *Apis mellifera* (Hymenoptera: Apidae)

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Abstract

The ectoparasitic mite, *Varroa destructor* (Anderson and Trueman) is considered one of the most serious pests of beehives, causing tremendous damage to honey bees, *Apis mellifera* L. and great economic loses to the beekeeping industry. Efficacy of six natural plant products (Pomegranate, Garlic, Marjoram, Harmal, Black cumin, Ambrosia) was evaluated against varroa mite and A*pis mellifera* 2018 season. The tested materials were applied in different methods of application: spray method, dusting and fumigation methods. Results showed that fumigation was more efficient than spraying while treatment as dusting of the powder of these materials recorded the lowest efficacy. Treatment with Pomegranate fumigation resulted the highest efficacy against Varroa mite followed by Garlic and Marjoram (94.6, 89.8 and 89.8 % reduction for adult infestation) and 85.6, 86.1 and 82.4% for brood infestation. Spraying with extract of Harmal showed the highest efficacy against Varroa mite (91.88%) followed by Pomegranate (91.06%), Black cumin 89.11% and Garlic 88.82 % reduction for adult infestation. Among the tested plant products used as dusting the infested bee colonies, powder of Marjoram, Harmal, Black cumin and Garlic recorded the highest efficacy against Varroa mite infested adult (94.67, 94.67, 94.42 and 93.15 Reduction %). All products haven't any effect against *Apis mellifera* and colonies treated with these materials gave a significant high honey productivity compared with control. Results indicated that using plant products in controlling Varroa mite through IPM program seem to be the ideal way for controlling this mite; hence these materials are available, safety to environment, selective to bees, easy in application and economically cheap.

Keywords: *Varroa destructor*, biological activity, natural products, *Apis mellifera*.

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Introduction

Honey bee colonies are subject to infestation by insects, mites and diseases. The ectoparasitic mite, *Varroa destructor* (Anderson and Trueman) is considered one of the most serious pests of beehives. Parasitism can result in a loss of up to 25% of adult weight, severe deformations of the wing and reduced longevity of worker and drone honey bees (Hussein et al., 2016). Colonies infested with this mite have significantly reduced worker bee populations and eventually die if left without controlling (Al-Waili et al., 2012; Ariana et al., 2002). The widespread use of synthetic acaricides has led to the accumulation of residues in beeswax, propolis and to a much lesser degree, in honey (Eshbah et al., 2018; Calatayud-Vernich et al., 2017; Nai et al., 2017; Bargańska et al., 2016; Calatayud-Vernich et al., 2016; Chiesa et al., 2016; Porrini et al., 2016; Sattler et al., 2016; Hussein & Mostafa, 2009; Hussein et al., 2002). The development of acaricide resistance in *V. destructor* populations and the spectra of the contamination of hive products provide considerable need to develop new treatment strategies that minimize the potential for the rapid development of resistance and the accumulation of residues. Natural products have potential as elements for varroa mite control because some of them are selective and have little or no harmful effects on non-target species. Many natural products known to possess various bio efficacies such as ovicidal, anti-feeding and bioactivities against various pests without any adverse effects on non-target species. and they are found to be highly effective against insecticide resistant pests (Benelli et al., 2018; Eshbah et al., 2018; Calo et al., 2015). Thus, the present studies were carried out to evaluate the efficacy of certain natural products on the parasitic mite *V. destructor* (adult and brood infestation) and their selectivity on *Apis mellifera* to find out one of the natural products maximizing Varroa control and minimizing the site effect on the honeybee colonies and environment, with minimal costs.

Materials and methods

Experimental honeybee colonies: Honeybee colonies (63 colonies) of the first hybrid carnelian bees, *Apis mellifera Carnica* were selected among the colonies of a private apiary at Shosha village 7, Minia Governorate. The experimental colonies were divided into 7 groups, each having three sub groups each sub group contains three colonies as replicates. Average strength of the tested colonies was 8 combs covered with bees housed in Langstroth hive and the experiment was repeated twice during season 2018 and the averages of the treatments were calculated.

Preparations products and application of the tested plant: Six plant natural products were evaluated against varroa mite infested the honeybee colonies represented in Table 1. Three features (extract, powder and original raw materials (used as fumigation) of the tested plant products were prepared as follows.

Extracts: The used part of the tested plant products (Table.1) were washed more once and left to dry under room temperature ($30^{o}C\pm2$). They were grounded in an electric mill and sieved through 0.5 mm sieve. Samples powdered plant material (1000 g) except

Garlic were blended in 70% aqueous ethanol and kept in dark container for 24 hours. The mixture of the dried plant material and the solvent was stirred for 30 minutes using a magnetic stirrer. The cured extracts were filtered firstly over clean cotton followed by a filter paper with anhydrous sodium sulfate. Then it as evaporated under reduced pressure using a rotary evaporator. The farmed concentrated extract was stored in glass containers and maintained in the refrigerator for the experimental tests.

Preparation of garlic extract: The Garlic extract prepared according to Vijayalakshmi et al. (1999) method; in brief 5 ml of (vegetable oil) was added to 85 grams of chopped Garlic and allowed to stand for 24 hours, then 950 ml of water was added and they mixed with 10 ml of soap. The mixture was filtered and stored in covered bottle until treatment. The extracts were used as 20 ml /colony from the previous extract.

Powders: The dried portions of the tested plant products (Table 1) were grinded and each properly mixed with powdered sugar (1 part of the tested product to 4 parts of powdered sugar to constitute 6 powdered products, in addition to powdered sugar which used as control treatment. The powder preparations were used through dusting often achieved before sunset. Each prepared powdered were applied as 40 gm/colony.

Table 1: English and scientific name of the source of the natural products.

English name	Scientific name	Family	The part used
Pomegranate	*Punica granatum*	Punicaceae	Peel
Garlic	*Allium sativum*	Liliaceae	Bulb
Marjoram	*Majorana hortensis*	Lamiaceae	Whole plant
Harmal	*Peganum Harmala*	Zyghophyllaceae	Seeds
Black cumin	*Nigella sativa*	Ranunculaceae	Seeds
Ambrosia	*Ambrosia martitima*	Compositeae	Leaves and flowers

The original raw materials (Fumigation): The dried parts of the tested natural plant products were used for producing a smoke through burning in smoker which used in smoking on the experimental colonies. Smoking of the experimental bees was done through fumigation. The colonies were divided into seven groups for obtained the effect of plant material treated with the plant products as smoke material for 60 seconds every week for one month the Varroa fallen on the sticky bottom board of hives (covered with a plastic sheet coated with raw Vaseline) were counted.

Evaluation the tested materials: Level of infestation on live bees was determined through randomly collection of about 50 bees /colony in a gar partially filled with water and few drops of detergent (Liquid soap). The collected bees were shaken for 30 seconds, then bee were filtered through muslin (8 to 12 mesh/ inch) to remove the bees, and the passed adults of mite were then counted (Komeili, 1988). This procedure was done before the application and after 24, 48 hrs; 7, and 14 days of the application and the treatment was applied new. Also, infestation of brood was determined at the same time of examination of the adult bees. One hundred of sealed brood worker per colony were randomly

chosen. The cell capping were removed by forceps and the pupae were picked up to examine for mites presence (Marcangel et al., 1992). Counting the number of dead mites was done through providing the bottom brood of each brood chamber with sticky white paper smeared with thin layer of Vaseline. Evaluation the efficacy of the tested natural plant products in controlling Varroa mite, with calculation reduction percentage of mite infestation by applying Henderson and Telton (1955) equation.

$$\text{Reduction (\%)} = \left(1 - \frac{T_a \times C_b}{T_b \times C_a}\right) \times 100$$

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

Assessment of the side effects of applied natural plant products on daily rate egg lying of the queens: The combs of the treated colonies were inspected at 4 days intervals for successive two days period. Daily egg laying rate was calculated by subtracting total number of eggs of the first day out of the total number of eggs of consequent day. Counting of the egg cells based on the method reported by (Kefuss, 1978).

Assessment of the side effects of applied natural plant products on larvae viability: Determination of pupation percentages was based on calculation the percent of open brood cells larva reached to sealed brood stage (Pupa stage). Pupation percentages was determined twice for each treatment period. Pupation percentages was determined through marking an area of brood cells (100 cells) of each experimental hive using transparent sheet (Hassan & Aly, 1998). The marked area was examined again after 5 days for measurement the number of cells reached to pupa stage and the larva viability percentage was estimated by applying the following equation

$$\text{Pupation (\%)} = \frac{\text{No. of capped cells (pupaed)}}{\text{The original No. of cells having larvae}} \times 100$$

Assessment of the side effects of applied natural plant products on worker longevity: Second day after the treatment, the half of comb cages were used for caging on a compact area (4 x 6 inch for each colony) of a sealed brood comb of the treated colonies, twelve days later, the caged area was inspected and the combs with their loads of the half comb cages having emerged bees were got out from their colonies. The drawn combs were brushed for removing the un-caged bees; one hundred of emerged bees were individually cached gently by forces through a hole of their cage and marked on their thorax with a pot of quick- drying paint having a particular color. All marked bees along with their combs were subsequently reintroduced to their respective colony by the swarm introduction method (Grout, 1960). The tested colonies were inspected in the dusk at 4 days intervals until the disappearance of the marked workers. The longevity was calculated as the midpoint between the rest day the bee was observed and the next observation date (Terada et al., 1975).

Assessment of the side effects of applied natural plant products on estimation of honey production: Honey

yield in experimental colonies were estimated by measuring open and sealed honey areas in June and September and transformed into weight using the following formula according to (Shawer et al., 1986).

$$\text{Honey yield in kg} = (\text{Area of honey (square inches)} \times 10.64)/1000$$

10.64 =amount in grams of honey in one square inch, based on averages calculated from unsealed and sealed honey from combs of different thickness.

Statistical analysis: The collected data were subjected to one way analysis of variance according to method of Mead et al. (1993). The statistical analyses were conducted using the SPSS 16 and F- test. Differences among means were determined by Duncan's multiple range tests.

Results and Discussion

Spraying application of the extracts of tested natural products: Results in Table 2 showed that treatments the infested colonies with dosage (20 ml/colony) of extracts of the tested natural plant products resulted in reduction of Varroa infestation by various levels. The highest average reduction percentage (91.88%) of adult bee infestation was recorded with spraying the extract of Harmal followed with Pomegrante (91.06%) followed by Black-cumin (89.11%) by the rate of 20 ml /colony and Garlic (88.82%) with significant differences among them as shown in Table 2. At the same time, treatment of the infested colonies with these natural products showed much variation in brood infestation. The average number of dead Varroa mite fallen was obtained when the colonies sprayed twice with the extract of Garlic (91.15%) followed by Black-cumin (86.57%) by the rate of 20 ml /colony. The differences among the reduction percentages of mite infestation resulted through treatment of these natural products from statistical view point were significant. Regarding the average % reduction on infestation after 24 hrs the treatment with different tested substances, the highest reduction % in the number mites was recorded for application of 20 ml/ colony of Black-cumin (78.84%) followed by Garlic (70.99%). Marjoram preparation gave the lowest one (59.17%) (Table 2). The statistical analysis of the obtained data showed significant differences among means of reduction percentages of mite infestation in the two treatments as shown in Table 2.

Table 2: Effect of spraying of extracts plant products on their infestation level of the parasitic mite, *Varroa destructor* after 14 days post treatments (General Average).

Treatments	1st spray Reduction of infestation (%)			2nd spray Reduction of infestation (%)			Average of Reduction of infestation (%)		
	Adult	brood	fallen varroa mite	Adult	brood	fallen varroa mite	Adult bees	Brood	Fallen mite
Pomegranate	93.27	77.08	36.67	90.48	62.50	101.50	91.88	69.79	59.34
Garlic	94.62	77.08	40.67	91.67	86.67	104.50	93.15	81.88	64.50
Marjoram	96.01	69.44	32.83	93.33	91.67	117.50	94.67	80.56	69.00
Harmal	96.01	88.54	36.00	93.33	84.62	115.50	94.67	86.58	69.50
Black- cumin	95.51	79.63	31.67	93.33	80.00	120.30	94.42	79.82	71.40
Ambrosia	94.95	69.44	24.50	85.19	83.33	107.80	90.07	76.39	63.99
LSD 0. 05	3.12	7.82	12.41	ns	10.61	17.30	-	-	

Dusting application of different powder concentrations of tested natural products: Reduction percentages of Varroa mite infestation on adult bees in Table 3. It was clear that treatment with Marjoram and Harmal (94.67%) followed by the Black-cumin (94.42%) followed Pomegranate powder by the rate of 40 gm/ colony resulted in decreasing the infestation by 91.88 %. by the rate of 40 g / colony. Also, mite infestation in the brood of the treated colonies suppressed by 86.58%, 81.88 % and 80.56 % for treatment with Harmal, Garlic, and Marjoram, respectively. The differences among general reduction percentages of mite infestation resulted through treatment of these plant products were significant (0.05%) as shown in Table 3. With looking to the number of dead Varroa mite fallen after 24 hrs of the treatment with different tested substances, the highest reduction % was 71.4% that recorded for application of 40 gm /colony of the Black- cumin preparation, while the lowest one was associated using Pomegranate preparation (59.34%). Significant differences among means of reduction percentages of mite infestation resulted by application of different substances as dust formulation.

Table 3: Effect of dusting powder of certain plant products on the infestation level of the parasitic mite (*Varroa destructor*) during the two spray and their average.

Treatments	1st spray Reduction of infestation (%)			2nd spray Reduction of infestation (%)			Average of Reduction of infestation (%)		
	Adult	brood	fallen varroa mite	Adult	brood	fallen varroa mite	Adult bees	Brood	Fallen mite
Pomegranate	88.46	84.72	36.67	93.65	80.00	89.25	91.06	82.36	62.96
Garlic	85.04	95.63	40.67	92.59	86.67	101.30	88.82	91.15	70.99
Marjoram	89.23	79.63	32.83	88.89	73.33	85.50	89.06	76.48	59.17
Harmal	93.27	92.36	36.00	90.48	80.00	102.00	91.88	86.18	69.00
Black- cumin	86.54	89.81	31.67	91.67	83.33	126.00	89.11	86.57	78.84
Ambrosia	85.64	69.44	24.50	87.30	73.33	96.00	86.47	71.39	60.25
LSD 0. 05	3.62	3.39	8.94	4.87	2.89	11.89	-	-	-

Treatment of fumigation of certain plant products during first season: Concerning the general reduction percentages of mite population data presented in Table 4 showed that the efficiency of the tested material for reducing Varroa population on adult bees could be managed in the following descending order Pomegranate > Black cumin> Garlic > Marjouram and Harmal > Ambrosia. The least effect was observed in treatment with Ambrosia. The average percentages of reduction were 94.6, 91.1, 89.83, 89.80, 87.9 and 83.5 % treatment by the rates of 40 gm/colony for the above-mentioned substances, respectively. General reduction of mite infestation in brood of the treated colonies showed the highest reduction percentage (86.10%) was obtained when the colonies treated with Garlic (86.10%), followed by Pomegranate (85.70%) by the rate of 40 gm/ colony, Black- cumin (85.10%) and Ambrosia (73.60%). Regarding the number of dead Varroa mite fallen (average of post-treatment with different tested substances, the highest reduction% (56%) when the colonies treated with Marjoram plant fumigation. While the lowest one (45.6 %) was associated using dosage 40 gm / colony of the Harmal

preparation. Statistical analysis indicated that there were significant differences among means of reduction percentages of mite infestation resulted by application of different substances in the two treatments.

Table 4: Effect of fumigation with certain plant products on their infestation level of the parasitic mite, *Varroa destructor* during two sprays with three concentration 40 gm/colony after 14 days post treatment and their average.

Treatments	1st spray Reduction of infestation (%)			2nd spray Reduction of infestation (%)			Average of Reduction of infestation (%)		
	Adult	brood	fallen varroa mite	Adult	brood	fallen varroa mite	Adult bees	Brood	Fallen mite
Pomegranate	97.00	91.27	18.5	93.65	92.10	80.00	94.6	85.6	50.40
Garlic	82.20	84.70	15.5	92.59	97.50	87.50	89.83	86.1	46.70
Marjoram	92.30	89.80	17.5	88.89	87.30	75.00	89.80	82.4	56.00
Harmal	89.23	88.54	17.00	90.48	86.60	73.30	87.90	80.9	45.60
Black- cumin	94.80	92.40	18.00	91.67	87.30	77.80	91.10	85.1	54.00
Ambrosia	89.20	84.70	14.00	87.30	77.80	62.50	83.50	73.6	46.80
LSD 0. 05	8.16	4.32	3.20	1.98	9.86	4.79	-	-	-

Selectivity of treatments with different method of application of natural products on bees: As shown in Figure 1, treatment colonies with 20 ml of extracts of certain plant products showed various effects on daily rate of egg. The average two sprays showed that the daily rates of egg laying were 655.00, 634.00 and 630.00 eggs /day for the queens of the colonies treated with Harmal, Garlic and Pomegranate respectively. In comparison to (287.50 eggs/ day) for the queens of untreated colonies. Larval viability the highest percentage (91.00%) was recorded with those colonies treated with Black cumin, with no different significant effect between treatments but there a significant effect between treatments and control. Larvae viability percentage of the brood of untreated colonies was (87.50%). Worker longevity revealed that the workers of the treated colonies lived various periods longevity of the workers belong to those colonies treated with the Harmal, Garlic, Pomegranate were 43, 37 and 34.90 days respectively in comparison to 21.13 days for the workers of the control colonies. The honey production of the experimental bees, data showed that the highest rate (10.34 kg/ colony) of the honey was produced by the colonies treated with the Garlic followed by those colonies treated by the Harmal (9.75 kg/ colony). Treatment with Pomegranate fumigation gave the highest efficacy against Varroa mite followed by, Garlic and then Marjoram. Spraying with extract of Black-cumin showed the highest efficacy against Varroa mite followed by Garlic and then Harmal. By dusting application powder of Pomegranate recorded the highest efficacy against Varroa mite followed by Garlic then Marjoram. Investigation on mode of action of essential oils or natural products chemical is important for mite control because it may give useful information on the most appropriate formulation and delivery means. Volatile compounds of many plant extracts and essential oils are composed alkanes, alcohols, aldehydes and terpenoids, particularly mono-terpenoids, and exhibit fumigant activity (Al-Waili et al. 2012, Ariana et al. 2002) indicated that aromatic plants and their essential oils

have been used as antimicrobial, acaricidal and insecticidal agents and to repel insect and mites or protect stored products. These constitute effective alternatives to synthetic pesticides without producing adverse effects or the environment (Isman 2006, Isman & Machial 2006).

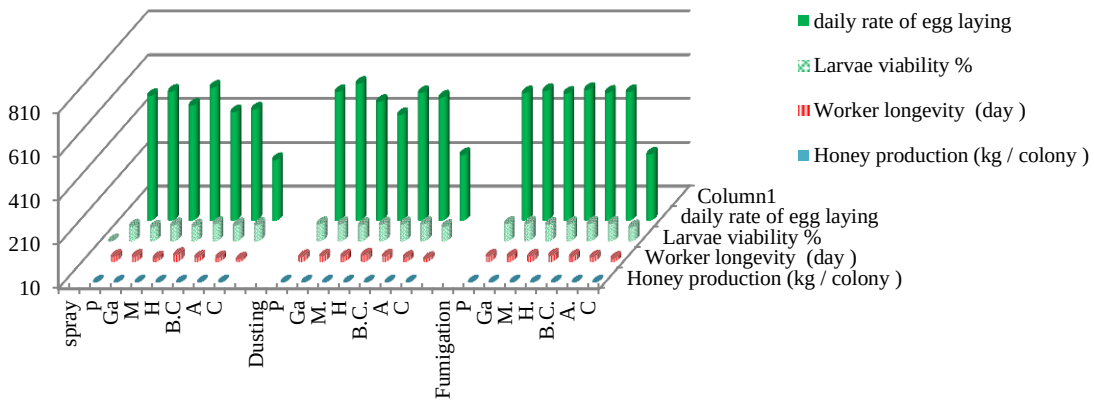

Figure 1: Effect of spraying, dusting and fumigation of certain plant products on daily rate of egg laid, larvae viability %, worker longevity and productivity of honey bees for season of 2018 (P: Pomegrnate- GA: Garlic – M.: Marjorum, H: Harmal, B.C.: Black cumin, A.: Ambrosia, C.: Control).

Moreover, the interest is natural products such as essential oils has regained momentum during the last decade primary due to their fumigant and contact acricidal activities and the less stringent regulatory approval mechanisms for their exploration due to long history of use (Isman, 2006). In general new alternatives for the control of *V. destractor* are necessary because of the rapid and widespread development of pyrethroid and organophosphate resistant mite population and because of the potential for contamination of hive products by these chemicals (Hussein & Mostafa, 2009). Natural products especially contain essentials, and especially components of essential oils, may serve as alternatives or as adjuvants to traditional treatment measures. From our results we can concluded that treatment the Varroa infested colonies with the different features of the plant products indicated that fumigations was more efficient than spraying while treatment with dusting of the powder of this materials recorded the lowest efficacy and all treatments were good selective against bees. Future research can assist in this effect by focusing on the characterization of the dose response relationships between components and mite/ bee toxicity and effects on mite behaviors.

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