

Fighting Varroa Destructor Parasitizing The Honey Bee "*Apis Mellifera Linnaeus*" Using Black Seed "*Nigella Sativa*" Infusion, and Neem Tree "*Azadirachta Indica*" Fruit Powder Infusion

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Abstract: The effectiveness of the black seed (*Nigella Sativa*) infusion and fruit powder infusion of the Neem tree (*Azadirachta indica*), the widespread in the Arabian Peninsula desert was tested, in fighting Varroa destructor on honey bees (*Apis mellifera*). The results showed variation in the number of destructor falling during treatment periods that extended to four consecutive weeks, the results were counted through calculation of the number of deaths at the end of each one of the four weeks, for each of the samples treated with both preparations, where the samples treated with the fruit powder infusion of the Neem tree (*Azadirachta indica*) recorded the highest level of the falling numbers during the third week, (112,5741), followed by the second week (85,2857), and then the fourth (33,2857), while the first week recorded the least proportion of the falling destructor due to treatment (28,5714). The samples treated with the black seed (*Nigella Sativa*) infusion the highest percentage of the falling destructor in the third week (135,7143), followed by the second week where the score was (73,5714), and then the fourth week (58,2857), and finally the first week that scored the lowest number of falling destructor, with an average of (32,8571), where it is likely to attribute the rise in the third week to the penetration of the material used in control in the bees, helping to get rid of the samples infected with *Varroa destructor* due to the penetration of the material used in the treatment in the parasite tissues, while the decline in the last week is attributed to the decrease of the numbers of the infected bees in the hives. We recommend using the black seed (*Nigella Sativa*) infusion, and the dried fruit powder infusion of the Neem tree (*Azadirachta indica*) in the fight against *Varroa destructor*, being two products safe to the beehive products, and their easy use throughout the year, and low economic cost.

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Key Words: Varroa destructor, *Apis mellifera* Linnaeus, *Nigella Sativa*, *Azadirachta indica*

1. Introduction

Varroa destructor is an external parasite of the mite types that feed on immature phases (larvae and pupae) and full phases of members of the honey bee *Apis mellifera* (worker, queen, male), and that parasite was discovered in Saudi Arabia for the first time in 1989 (*Al Ghamdi and Hoopingarner, 2002*). The Varroa infection causes the emergence of bees with deformed wings, decrease in bee worker weight, shortening its age, as well as the death of a large proportion of the brood, the incubator would emerge perforated, and the death of a large number of members of the community, leading to the destruction of the community, and the symptoms characteristic of infection with parasite varroa, is the presence of bees with deformed wings, abdomen and sometimes legs also, and observing the parasite with its distinctive shape on the adult bees bodies, and the incubator infected would show perforated and irregular in appearance, and the beekeepers fight *varroa destructor* and forcing its population to be less than the level that causes damage to their beehives, and beekeepers use different materials in control, including chemicals, such as Ibustan, Bayevarol, Mavrick, Folpeix,

Malathion, and others, where the extensive use of pesticides and chemicals had led to wide range of the negative effects resulting in hazardous residues in hive products of honey, royal jelly, wax, pollen, and others (*Zakaria and; Figen et al., 2012; Vimla et al., 2013; Sammaturo 2000; Ruffinengo et al., 2007*).

Recently, many research and studies were published on the use of natural materials such as plant extracts and essential oils in the fight against bees destructor, where plant extracts and essential oils are inexpensive and have no damage to public health, as they are consumed by human, and are at the same time safe for bee products even if used for a long period of time, and aromatic volatile oils were used in the fight against Varroa mites, such as peppermint oil, *Thymus vulgaris*, *Origanum majorana* oil, *Rosmarinus officinalis* oil, and *Artemisia jordanica* oil, by making sweet cakes blended with volatile oils for the bees, so that Bee body becomes saturated with oils causing Varroa mites suffocation and causing damage to its respiratory system and its death, and also by performing the control process using the filtrate in the hives, which takes place by putting a piece of adhesive paper inside the Frankish cells on the hive bases below

the disks (sticker paper cannot be put inside the cells below disks) taking into account to put a wire grid on top of the sticker paper to protect the bees from getting stuck. Vegetable extract may be used also of each of garlic and camphor in the control against Varroamites in honey bee colonies by boiling a little of garlic with eucalyptus leaves in a little water, then filtered and to add to the solution two tablespoons of sugar, then the solution may be sprinkled on the bees for 3-4 days. In this study, the black seed *Nigella Sativa*, and neem tree *Azadirachtaindicadry* fruit powder were used in preparation of confections to combat Varroamites in infected hives (*Nouraldin and. Alburaki 2015*).

2. The Method

Collection and incubation of the mites

The mites were collected from a number of separate colonies, using converted carbon dioxide method, and then, in this experiment bees swarmed with mites were collected in a box with a screen in the bottom of the container, Rubbermaid, then moving the container at 400 rpm for 10 minutes in an orbital form, during exposure to carbon dioxide.

The mites fell in the bottom of the container and then collected by a soft brush, and put in Petri dishes, that were lined with wet bath paper, and then the mites were entered into small beehives by fine-tipped brush through a wire mesh screen serving to cover hive roofs and preventing the bees from flying out, and then putting the mites directly on bees.

Rating of Mites and Bees Mortality

To assess the population groupings of bee colonies at the end of the experiment, colonies were visually scored from both the upper and lower sides, where the number of tiers apparent, while completely covered with bees, that were counted for statistics (each tier has about 2430 bee workers, to monitor the mortality of bees and the mites, where a white poster board of an area of 16.61 cm was completely covered with wax paper and put in the bottom of each hive,

where bee workers and mites would fall down outside the hive, average of varroamites available was counted through recording the number of the mites on each bee.

In each hive, the count took place on the last day through the collection of adult bee workers, that was as 200-300 bee workers, and alcohol washing technique was used to remove the mites of the bees (*Gatien and Currie 2003.*)

Mites were removed of the dead bees according to the method of collecting the bees in 70% ethanol for ten minutes using *Labline Orbital Shaker* and rotation at 200 rpm.

After shaking the bees, the basket containing the bees is removed, and counting the mites falling of the bees and in order to make sure of the numbers of the mites found in every group of the experiment.

The compound of black seed *Nigella Sativa*, and the dried fruits of the Neem tree were prepared and applied to honey bees to control the Varroaby preparation of the vegetable extract for each of the black seed *Nigella Sativa* and dried fruit powder of the Neem tree, *Azadirachtaindica*.

About of kg of both of the above materials were weighed for extraction and then soaked in a pot with enough quantity of water to cover the amount of the plant material, and then heating little by little to pre boiling point, and then the plant material was moved with the water to large dark glass bottles for 24 hours, then filtered by a piece of cloth, and plant material is disposed of, and the filtrate only taken.

The study was conducted in the spring, in Al Huda area in the city of Taif, located on the highlands in the western part of Saudi Arabia, for four consecutive weeks.

Falling mites were collected every day, and analysis was made for each week separately.

Statistical analysis:

T-test, version 21

The result according to the following tables:

Table (1): The number of Varroa destructor fallen by the treatment with black seed, *Nigella Sativa* infusion, of the bees in each week of the four-week experiment

		Mean	Correlation	Std. Error Mean	Sig.
Pair 1	black1	32.8571		0.38237	.000
	black2	73.5714	.987	0.69476	
Pair 2	black1	32.8571	.951	0.38237	.001
	black3	135.7143	.	0.90566	
Pair 3	black1	32.8571	.972-	0.38237	.000
	black4	135.7143		0.52066	
Pair 4	black2	73.5714	.953	0.69476	
	black3	135.7143		0.90566	.001
Pair 5	black2	73.5714	-.987	0.69476	.000
	black4	16.1429		0.52066	
Pair 6	black3	135.7143		0.90566	
	black4	16.1429	-.968	0.52066	.000

Table (2) Numbers of Varroa destructors falling due to the treatment with Neem tree;Azadirachtaindica, dried fruit powder infusion, on each of the experiment four weeks.

		Mean	Correlation	Std. Error Mean	Sig
Pair 1	gree1	28.5714		0.76879	.000
	green2	58.2857	.991	0.05741	
Pair 2	gree1	28.5714	.975	0.76879	.000
	green3	112.5714		0.88289	
Pair 3	gree1	28.5714		0.76879	
	green4	33.2857	-.972	0.32175	.000
Pair 4	green2	58.2857	.979	0.05741	.000
	green3	112.5714		0.88289	
Pair 5	green2	58.2857	-.992	0.05741	.000
	green4	33.2857		0.32175	
Pair 6	green3	112.5714		0.88289	
	green4	33.2857	-.957	0.32175	.001

Table (3) Comparison between the falling numbers of Varroa of the lab bees treated with Black; Nigella Sativa seed infusion, and Azadirachtaindica

		Mean	Correlation	Std. Error Mean	Sig.
Pair 1	black1	32.8571	.969	0.38237	.000
	green1	28.7143		0.68897	
Pair 2	black2	73.5714	.985	0.69476	
	green2	58.2857		0.05741	.000
Pair 3	black3	135.7143		0.90566	.002
	green3	112.5714	.941	0.88289	
Pair 4	black4	16.1429	.992	0.52066	.000
	green4	33.2857		0.32175	

4. Discussion

Experiments showed that the use of the black seed; Nigella Sativa preparation and the Neem tree; Azadirachtaindica treeb fruit powder infusion preparation, and its effectiveness in controlling the pesticide of Varroa destructor, although the Neem tree; Azadirachtaindica fruit has shown higher efficiency to overcome the scourge of Varroa destructor compared with the Black seed; Nigella Sativa, and in previous studies, using natural products as aniseoil, in different ways, such as vaporization, distillation had shown good effectiveness in the fight against Varroa, safely and in ways easier in the application, minimizing damage to the environment and reduction in the economic cost to the owners of the apiaries affected (*Le Conte et al, 1998; Qayyoum et al, 2013.*)

This study showed an agreement with the results obtained in the studies conducted (*Kraus, 1990*) using natural preparations (anise) where high effectiveness in the fight against Varroa destructor was recorded in brood and bee workers and adults respectively.

The results of some studies confirmed that the oils extracted from the fruits of anise have higher effectiveness through affecting by touching, and may

be due to the high effectiveness of anise seeds to the extruder influence, which confirmed by (*Kraus, 1990*) in tests to assess the effect of essential oils of many plants and may be attributed to this reason, the high effectiveness of preparation of the Neem tree; Azadirachtaindica, fruits, in causing death of the mites, more than that in preparation of black seed; *Nigella Sativa*, infusion.

No negative effects have been observed on the bees and brood as a result of the control with both black seed Nigella Sativa infusion, and Neem tree; Azadirachtaindica powdered fruit infusion, as were applied by observing the populations treated on an ongoing basis, and the both of these two preparations are safe to bees, which was confirmed by this study and earlier studies conducted on other natural preparations, (*Daher-Hjajj and Alburaki, A. 2006, Abd El-Wahab et al 2012, DeGrandietal, 2012.*)

It was observed that there is great increase in bees and brood coverage in the treated populations compared to non-treated ones.

The black seed; Nigella Sativa infusion and Neem tree; Azadirachtaindica, fruit powder infusion has safe vegetable source on the beehive products and

its importance lies in being of a distinctive effectiveness, as shown by the study, in the fight against the Varroa parasite, as well as for ease of application despite the need of this method for further study by researchers on different breeds and in different climate areas.

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