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EFFECT OF NATURAL BIOPOLYMERS (POLYMER AND THYME OIL) AND POLYMER AGAINST *VARROA DESTRUCTOR*OUD (ANDERSON) AND ON THE VITALITY AND ACTIVITY OF HONEY BEES *APIS MELLIFERA*

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ABSTRACT
The study was conducted in the laboratory bees in the College of Agriculture - Wasit University at the period from 1/4/2016 to 11/2/2017. The polymer and polymer with oil were tested on the death rate of *Varroa destructor* of infected worker bees, the death rate was highest reached 2.468% individuals when the polymer with thyme used compared to the treatment of polymer alone and control, which reached 1.332 and 0.066% respectively within 72 hours. The results showed the cells treated by biological polymer gave best biological and life characteristics of honey bees in terms of area (eggs, brood, honey, and pollen), additionally to increase the weight, the number of operators bees and less space for the empty sixth eyes compared to polymer alone and control treatments.

KEYWORDS: *Apis mellifra, Varroa destructor*, worker bees, infection.

INTRODUCTION

For many thousands of years, the pharaohs have cared for the raising of bees in cylindrical clay hives. The bees were from the sanctuaries, and engrave on many of their remains (Hijazi, 1998). Since ancient times, the human has known honey as food and medicine, where the beekeepings appeared and use it in agriculture and for obtain its products and trade (Ramal, 2005). Therefore, many specialized medical centers have been established for the treatment of bee products worldwide, as in Russia, China, Japan, Europe and America (Hilal, 2003). The honey bee pollutes about 71% of the 100 most used plants, which account for 90% of the world's food and is exposing to many of pesticides in the stages of its development, either by bringing it in pollen and nectar or because exposed to many diseases and pests, such as the parasite *Varroa destructor*. The absorption and adsorption agents of chemical materials used to pests can be affected on the bee and its hives; about 170 different pesticide residues of beeswax have been isolated in American hives including insecticides, Herbicide, and fungicide from the pollen environment. The good beekeeper can reduce the presence of these pesticides by preventive and curative methods (Spivak and Reuter, 2016 and Johnson, 2010). Pollinating insects are bio-agents to environment system that have the greatest role in keeping biodiversity and supporting agricultural crops. Honeybees are the most important bio-pollinators. Although bees are exposed to non-lethal doses, they may not die but their behavior is affected (Rain and Gill, 2014). About 30 insecticides were identified within the honey bee hives (Johnson *et al*., 2010). As a result of the spread of the *V. destructor* and greater wax worm *Galleria mellonella* in most of Iraq in the hives and stores for most of the year to suit the environmental conditions. Recently, the polymer control - released polymers pesticides is one of best methods involving with pesticides to control many types of economic and domestic insects. The technique relies on making the active material of the pesticide in the form of microencapsolation (Tagadin, 2000). The importance and the purpose of this study to reaching methods modern control non-effect on of humans, bees, and the environment as alternatives to chemical pesticides, which may be integrated with each other, the research is looking at the improvement and development of controlled polymer preparations to *V. destructor*.

MATERIALS & METHODS

Create the hives

Nine hybrid colonieswere chosen (Iraqi strain) *Apis mellifera* L. each colony contained (5) frames. They were selected to be the same denominations by force, and queens from one strain and one year old. Thehives and frames were from one type of wood in the same form and measurements, free from diseases and pests to reduce or avoid the negatives and differences that affect the activity and effectiveness of biological inside or outside of the hive. The hives were balanced in brood areas, pollen and honey. The new hives were closely in the weight (after being converted into new and similar hives). Then, transferred to modern Lancaster hives regarding manufacture (new) and the quality of wood and shape. Frames were added to the hives when they needed. The hiveswere randomly distributed and labelled for each treatment.

Extraction of essential oils for thyme and calptus (Clevenger machine)

Extraction process oils were in Medicinal Plants Research Unit - College of Agriculture, Baghdad University by using Alclavngermachine.Thyme andcalptus plants were purchased as a plant from local markets and collected the leaves of the plant from Wasit city, and the plants were diagnosed in the College of Science –Baghdad University. The leaves were spread on the floor of the laboratory was stirred daily until they dried. The leaves were ground by the mill with the speed of 2500 cycle/min. The extraction process was immediately after grinding so as not to lose the active ingredients (essential oils), the most volatile as it has been flooded papers for oil extraction them completely with distilled water into a beaker and raise the flask temperature to less than the boiling point of water (85°C). To be evaporation and condensation process, to maintain the quality of the recovered material was very careful to control the temperature and time required for exposure to heat source commensurate temperature with this type of oil extracted, the water in the beaker acts as kept from overheating. After the increase of water loaded with aromatic oil to the top was intensified by the water-cooled solenoid tube and thus combine the condensed steam with the essential oil inside the distillation device and after cooling the vaporizing material condenses and separates oil from water through the inserted tube and collects essential oil through the valve, Fine Therm was used for that.

Polymer preparation

The polymer preparation was as followed: consisting of maize, glycerol, beeswax, vegetable oil (thyme or kalitos) and 80% tuna. Then, prepared with 350 mL glass container containing distilled water (water bath) was placed inside 250 mL glass container (Baker) containing 80 ml of distilled water and placed on the Hotplate Stirrer Rlabinco Model L-81.(After weight of the second baker with the magnets of the device),and waiting for distilled water to reach 45^{o}C, the starch was added gradually (5 g per 100 mL distilled water) with the rotation speed of the magnet 100 cycle/min and the temperature at 75°Cto minimize evaporation and wait for 30 minutes. Then, the cholesterol was added 30% from starch at thesamespeed and temperature after 30 mins the beeswax was added 10% and decreased the temperature to 60°C. After that, 0.5,1 and 1.5%g/L concentrations of thyme and salptus oil were added for 30 mins. Twain 80% was added like a half of wax weight and complete the full level by distilled water to 100% and waiting for the polymer was cooled and sprayed and the required quantity was prepared according to the different treatments (Aliabadi *et al*., 2013).

Create *Varroa destructor*

V. destructor mites were obtained by searching for hives infected and were non-treated by pesticides against mites during the previous season.

In *in vitro* examination, to detect the effect of different treatments on varroa mites have been worked wooden boxes, the base, lid, and sides were dimensions (15 × 15) cm. The dimensions of the front and rear facades were (13 × 14,7) cm, and inside it is a wooden frame of 12×11 cm contain on wax in all its size. The box contains a hole along the base height of 3 mm to allow the adhesive paper to be placed inside the box with dimensions of 10 x 13 cm for sticking the fallen mites upper cover of the box can be turnover and It has a lock, its bottom part contains cloth buckle (used in windows to prevent flies). The small box was equipped with a pollen and a piece of honey containing water, it was replaced daily.10 bees infected with mites parasite (each bee infected by one a mite) and 20 bees healthy were placed in each treatment and each replicator.

Treatment of wax, box, infected and healthy bees (therapeutic dose)

The infected bees were infected by larvae of *V. destructor*, the contents of the box and its sides were sprayed with the treatments that gave the highest concentration results (polymer and thyme oil only 1.5 g and polymer) and control treatment as a comparison was sprayed with sterilized distilled water only in the same manner as mentioned.

Effect of different treatments on internal and external bee activity

Measuring of brood comb area

Brood combs area were measurement for each hive (workers and males) inch ^{2}(Alnaji, 1980., Jeffree, 1958) where useda transparent plastic frame divided into squares (the area of each square is 1 inch^{2}),by placing the brood frame and measuring the area of the plastic frame, and each side of the frame and conducted the test at the beginning of the experiment before the conduct of treatments and at the end of the experiment to show the differences between them.

Measuring of honey comb area

The honeycomb was measuring in hives were treated as in table-2.

Measuring of pollen comb area

The pollen comb area was measuring in hives were treated as in table-2.

Measuring of eggs area laid by the queen

The eggs are awas measuring in hives were treated as in table-2.

Measuring of activity of scout bees

To detect the effect of field treatments on the activity of scout workers in fields, the number of scout workers was measured after one day of treated in the morning, the number of workers (entering to hive) for one hour and one reading rate per hour (9-10) was randomly calculated using precise digital cameras where it was fixed near the hive door.

Total growth of treated hives

To study the effect of treatments on the overall growth of hives, the test was conducted in two ways, as follows:

Measure the number of frames in hives

The number of frames in each hive were calculated (the original number of frames in each hive at the beginning of the experiment was five frames per hive and in all treatments). The number of waxy frames added to each hive was measured as needed, by calculating the total number of frames at the end of the experiment for the hives; we obtained the growth in each hive and compared that with the number of frames in the control treatment of hives.

The rate of increase in the overall weight of hives

The hives have been weighed for detecting the effect of different treatments on the overall growth of hives, the

process of the weighting of wood hives (without bees) and the process of weighting of the wood hives in all parts including bees, broods, honey and pollen at the end of the experiment to show the amount of increase in the treated hives and compare them with control treatment.

RESULTS & DISCUSSION

The results showed (Table 2) the superiority of the treatment of polymer and thyme oil by giving it the highest death rate of *V. destructor* of 2.468% and a significant difference from the polymer treatment which reached 1.332% and compared with control (Distilled water) reached 0.066%. In the first hour, no significant differences between the treatments, and may be due to reduce the activity and effectiveness of polymers in this short time. The results showed (Table 2) the superiority of the treatment of polymer and thyme oil on polymer and control treatments in all subsequent times due to the effectiveness of the bio-polymer and release amount of oil, which has the largest role in the killing of *V. destructo*r. These results are consistent with previous results, Finley and Sammataro (2007) described use of thymol to control pests which recorded in EPA Code 080402, which evaporates depending on the temperature after being placed in a carrier (gel) and thus spreads in the hive and moves from one bee to another by licking of larvae and then to all hive. The results were consistent with the effectiveness of the starch polymer when used alone, Ali and Mohammed (2008) used several compounds, including oils, powder, liquid, emulsion, pastries, capsules, steam or any other form such as dextrin, maize, Arabic gum, cellulose, hydroxypropyl methylcellulose and other which have proven to be effective for several months according to the concentration used, these substances have not affected on the life, vitality and activity of bees, and it slowed the growth of the mites or prevented the production of new individuals.

TABLE 2: Effect of direct spraying of infected bees by different treatments *in vitro*

Treatments	Death rate / hours					Rate
	1	8	24	48	72	
Plymore+thyme oil 1.5 g/l	1	1.67	2.33	3.67	3.67	2.468
Plymore	0.33	1	1.33	1.67	2.33	1.332
Distilled water	0	0	0	0	0.33	0.066
L.S.D.	N.S	0.666	0.942	0.942	1.153	1.007

The results of Table (7) showed no significant differences between the cultivars involved in the experiment for all the traits to be measured. The treatment of polymer + thyme was characterized by giving the highest area of eggs at 29.1 in² and a significant difference from the polymer treatment alone, which reached 18.9 in², the number of eggs decreased before the treatment because of high temperatures after the end of the experiment and the start of the season of honey.

The area of brood showed the treatment of polymer+thyme reached highest area of incubation 108.5in², which did not differ significantly from the treatment of polymer alone, which reached 73.5in² compared with control treatment reached 22.1in². Polymer + thyme treatment lead to evolved rapidly and exceeded the number of brood in control hives due to the good health of the treatments hives as a result of the elimination of the parasite. Hamaad and *et al.* (2008) pointed when used the thymol 100%, cinnamon, and lemon leaves (50-100%) treatments were sprayed on V. destructor and bees lead to increase of brood area reached 199,8،229,9 in², 218,6،229,9 in² and 199,6،210 in²respectively, compared with control treatment was reached 39.6 in². Thyme+ polymer treatment gave the highest honey area reached 21 in², which did not different significantly from polymer treatment, which reached 15 in² compared with control treatment which reached 5.2 in², may be due to the number of workers bees and the force of the active role in the collection of nectar.

Treatments	Area / in^2												Number of bee
	Eggs		Broods		Honey		Pollen		Empty		The weight		
	before	after	before	after	before	after	before	after	before	after	before	after	
Plymore+thyme oil 1.5g/l	45.8	29.1	54	108.5	1.87	21	1.07	13.6	91	33	5.55	13.8	535
Plymore	38.5	18.9	56	73.5	4.13	15	2.4	3.4	86	37	5.5	12.57	369
Distilled water	31.3	6.5	128	22.1	4.95	5.2	2.3	3.1	102	453	5.75	11.03	155
L.S.D.	N.S	12.91	N.S	52.95	N.S	7.52	N.S	8.44	N.S	205.7	N.S	1.66	270.4

increase in the number of eggs and the number of brood

TABLE 2: Production with different treatments

The area of the pollen was up-regulated rate by thyme + polymer treatment, which reached 13.6 in² compared to polymer and control treatments, which reached 3.4 in²and 3.1 in², respectively, due to the increase in the area of the number of workers to collect pollen and storage.The treatments of thyme + polymer and polymer provided less empty space of wax frames reached 33 and 37in²respectively, indicating the activity and storage capacity of the components of the total hive compared to control treatment reached 453 in².For the total hive weight, the treatment of thyme + polymer gave the largest weight of hive 13.8 kg, which was no different from polymer treatment, which reached 12.57 kg, these results accordance with Hamaad et al. (2008) where showed the hive weights reached 3.4, 4 and 4.3 with thyme, cinnamon and lemons leaves treatment respectively, compared with

control treatment reached 3 kg. For the number of workers bees, the treatment of thyme + polymer showed the highest number of workers bees reached 535 bees and followed by polymer treatment reached 369, compared with control reached 155 bees, this result indicated to the extent of stimulating and activating the worker bees on sores and search.

REFERENCES

Aliabadi, S.S., Hedayat Hosseini, Mohammad Amin Mohammadifar; Abdorreza Mohammadi; Mehran Ghasemlou; Seyed Mahdi Ojagh; Seyede Marzieh H.and Ramin Khaksar (2013) Characterization of antioxidant-antimicrobial -carrageenan films containing Satureja hortensis essential oil. International Journal of Biological Macromolecules 52: 116–124

Jeffree, E.P. (1958) A shaped wire grid for estimating quantities of brood and pollen in combs. Bee World 58(3):105-110.

Sammataro, D. and Finley, J. (2007) The developmental changes of immature African and four lines of European honey bee workers, Apiacta 42: 64–72.

Probasco, Gene and John I. Haas (2008) Compositions and Methodes for Controlling A Honey Bee Parasitic Mite Untied States Patent (10) Patent N0: US 7,597,912 B2.

Hamaad, R.F.M., Eldoksch, H.A., Abdel-Samed, A.M. and Abdel-Moein, N.M. (2008) Effect of essential oils anda thymol formulation for controlling *Varroa destructor* in honeybee (*Apis mellifera*) colonies. Egypt. J. Agric. Res., 86(3): 951-961.

Johnson, R.M., Ellis, M.D. and Mullin, C.A., Frazier, M. (2010) Pesticides and honey beetoxicity USA. Apidologie 41: 312–331. doi: 10 .1051 /apido/ 2010018.

Spivak, Marla and Gary, S. Reuter (2016) Honey Bee Diseases and Pests. University of Minnesota St. Paul, Minnesota, Department of Entomology and Minnesota Extension Servicepp.42.

Gil, Richard J. & Raine Nigel E. (2014) Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. Functional Ecology British Ecological Society /1365-2435.12292.