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# THE RELATIVE EFFECTIVENESS OF BIOPOLYMERS (POLYMER AND THYMUS OIL) AND POLYMER ONLY ON PARASITE *VARROA DESTRUCTOR OUD* (ANDERSON) IN HONEYBEE HIVES *APIS* MELLIFERA

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**ABSTRACT**
This research was carried out in the laboratory of bees, College of Agriculture - Wasit University. Wasit on 1/4/2016. The experiment was conducted to demonstrate the relative effectiveness of varroa control methods by biopolymers within hives which is infected by the parasite. The results showed that the rate of kill for bio polymers (polymer and thymus oil) were significant differences with others, which gave around 94.94 varroa during 24 days. Biopolymers were characterized by providing high drop for varroa from the first days of treatment which was 86.49 compared with polymer treatment which gave 48.93 and 57.75 killing rate respectively. For the varroa number into brood and on the adults after the experiment was less number of polymer treatment and thyme oil and polymer only. These treatments were not significant differences compared with control, which gave 10.33 and 7.67 parasite on brood and adults respectively.

**KEYWORDS:** parasite, varroa control, biopolymers, thyme oil.

## INTRODUCTION

The infection of bees with varroa not causes the weakness of bees only, but the weakness of resistance to insecticides (Habbruge, 2006). Nicotine pesticides were one of the main reasons for the disappearance of bees (C.C.D), in addition to hunger and varroa infection (Henry *et al*., 2012).The using of chemicals is one of the most effective methods for varroa control, but the unregulated and excessive which gave varroa resistance to pesticides and pollution of bee products (Baxter *et al*., 1998). Marinellia *et al*. (2006) confirmed that it is impossible to eliminate the mites and reduce its spread within the hives without followed therapeutic and within reasonable limits for control it. Bees depend on cooperation and the involvement of their members in group work that all individuals are poisoned (Bryden *et al*., 2013). Insecticides are directly or indirectly involved in reducing their numbers and other benefit insects. If they do not die, they are changed their behaviour (Rand *et al*., 2015). The high intensity of the mites in the hives lead to damage to the tissues of bees, limit the proliferation of bees,the death of pupae, distortion of wings, legs, and abdomen and reduce in the lifetime of workers (Smith, 2008). Hejeej (2003) said that varroa is characterized by the rapid proliferation and spread, causing the destruction of bees in hives, which that the parasite occupies the first rank at the level of the Arab countries in terms of importance between diseases and pests most harmful to bees, according to the questionnaire of the Arab Organization for Agricultural Development. When the varroa entered to Iraq, they cause a huge damage Sieq *et al*. (1990), which confirm that the parasite was transferred some of viruses like a wing bomb (DWV) to pupae and adults of bees (Katherine, 2012). Several attempts have been made to reduce the damage and spread of varroa by using pesticides or natural products (Calis *el al*., 1999). As a result of the widespreadspread of the parasite, and to develop novel safe methods for humans, bees and environment as alternatives to using chemical pesticides, which can be integrated with each other like control-released polymers pesticides. This study will focus on improving and develop polymers with controlled released, which is an important research in current time. This is for control several of economic insects. This technique depends on to make the active material for pesticide like Microencapsulation (Taj Eddin, 2000).

## MATERIALS & METHOD

### 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 thymus and eucalyptus (Clevenger machine)**

Extraction process oils were in Medicinal Plants Research Unit - College of Agriculture, Baghdad University by using Alclavngermachine.Thymus and eucalyptus 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. Then, 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 (thymus or eucalyptus) and 80% tuna. Then, prepared with 350mL 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 (5g/100 mL distilled water) with the rotation speed of the magnet 100 cycle/min and the temperature at 75°C to minimize evaporation and wait for 30 minutes. Then, the cholesterol was added 30% from starch at the same speed and temperature after 30mins the beeswax was added 10% and decreased the temperature to 60°C. After that, 0.5,1 and 1.5%g/L concentrations of thymus and salptus oil were added for 30mins. 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).

**Prepared *varroa destructor***

Effect the different treatments of parasite of varroa

Varroa mite was obtained by searching for hives infected with varroa and no pesticides were used against varroa during the previous season.

**Field experiments**

For the applying the treatments that gave the best results and to show their effect, they have been implemented in thefield using the biological polymer (polymer and thymus oil 1.5 g / L), polymer only and control 5.

**Treatments were used in the hives include**

1 First treatment: polymer and thyme oil 1.5 g/L

2 Second treatment: polymer only

3 Third treatment: control (spread with distilled water only), in each treatment, we used three hives.

4 Measuring the relative effectiveness of field treatments

For confirm the relative effectiveness of the treatment (polymer and thymus oil, 1.5 g/L and polymer only), and the comparison treatments on the varroa parasite, the hives were transferred into new wooden hives and labeled them. Then, spray the hives from the inside (the base, box, the inner and outer cover of inside and the frame containing the brood and bees) with the above treatments the hives were provided with doors to prevent the entry of pests, including the red wasp. Spraying was done only once. Spray included complete coverage of the contents of the hive from inside using 500 mL for each replicate and by three replicates per treatment and by hive per replicate. We calculated the number of varroa daily for 25 days and replaced the paper when needed. At the end of thee xperiment, we estimated the number of varroa, which was attached the adults by choose 100 adults and 100 brood. Also, we estimated hive weight, honey, pollen, brood and eggs.

Measuring the relative effectiveness according to:

The intensity of infection in the sample:-

$$TX = \frac{NV \times 100}{NA}$$

TX means intensity of infection

NVmeans number of varroa

NA means number of bees of brood

The relative efficiency of each material and concentration was calculated according to the following equation (Hajij and Al-Baraqi, 2015, Hajij *et al*., 2014).

$$\text{relative efficiency } \% = \frac{\text{Varroa drop after control} - \text{normal drop before control}}{\text{Varroa drop after control}}$$

**Statistical analysis**

The results were analyzed using the ANOVA Table. The results were compared using the least significant difference (LSD) level ($p \leq 0.05$) for the comparing the results (Sahuki and Heib, 1990).

**TABLE 1:** relative efficiency% for different field treatments on varroa parasite

| | Treatments | | | |
|---|---|---|---|---|
| Time/day | thymus oil polymer | Polymer only | control | average |
| 1 | 70.56 | 0 | 6.96 | 25.84 |
| 2 | 81.3 | 21.67 | 32.99 | 45.32 |
| 3 | 90.96 | 58.81 | 78.42 | 76.06 |
| 7 | 88.47 | 58.33 | 65.72 | 70.84 |
| 14 | 92.72 | 73.49 | 78.00 | 81.40 |
| 24 | 94.94 | 81.27 | 84.42 | 86.88 |
| LSD | 10.32 | | | 5.962 |
| Average | 86.49 | 48.93 | 57.75 | |
| LSD | 2.10 | | | |

Table 1 showed that the relative efficiency was the highest value after 24 days of treatment, which was around 86.88%, which was not significantly different from the previous day at 14 days 81.40, while the relative effectiveness of the drop of varroa was the first day of the treatment which around 25.84. while for the treatments, the results of the table 5 showed that polymer and thymus oil where significant differences in relative effectiveness which gave 86.49 compared to the treatment of the polymer only, which gave 48.93. The reason for the effectiveness of thymol, which is huge part of thyme oil, also it has a role of repellent and killer of the varroa within the system where the polymer is mixed completely with thymus oil ( Mikityuk Grobov ,1979).

The combination of different treatments and time was characterized by treatment of polymer and thymus oil where they gave a relative effectiveness of 94.94 in day 24. It indicates the stability of thymus oil in the polymer and the length of its effectiveness and this is consistent with Chiesa (1991) which used thymol against Alvarwa for two seasons and gave the results of the killing around 96.77 and its effectiveness was increased by adding sugar based on temperature. It is explained by Lange (1990) that the system of the retina in which the polymer is fully mixed with the active substance, and the process of decomposition which leads to the exit of the active substance in successive layers and the idea of the launch is controlled by the fact that it expresses. The release of substances with biological efficacy was over a longer period than the standard time of work. Usually in The controlled release, the system is designed for polymer systems that control the release. The controlled release mechanism includes the presence of the active substance, whether it is a pesticide or another in the polymeric formulation, and therefore the active ingredient is released at relatively constant rates and at intervals that may be short or long nature of the active substance. And the method of association and the process of dissolution and the addition of vegetable oils with polymer have been weakened by the strength of the polymer tangle and gave the property of the length of his age and his birth, and this is agreement with (Astm, 1999). The permeability of water from the polymer or permeability of ketosan increased after the addition of thymus oil or basil because it has grown the spread or irritation or mobilization of volatile oils and overcome the strength of bonding and thus reduced the strength of the sclerosis of the film and this explains the effective strength of thyme oil and agrees with Aliabadi *et al.* (2013).Polymer modification by adding beeswax has improved its properties and is consistent with Fabra *et al*. (2009). While the used of 0.25g of thymol evaporation for each vacuum hive gave a killing rate of 55% while the Bauder gave 66-98%, although slight differences but very excellent in control (GLiebigi, 1995). Followed by the same treatment on day 14, which did not differ significantly, but significantly different from the treatment on day 2 and day 1 of the treatment, where it reached (81.3, 70.56) respectively. The treatment of polymer and thymus oil were significant differences compared with (polymer and comparison) treatments at all days. The relative efficacy was 94.94, 81.27.84.42) respectively for polymer, thymus oil and polymer alone and control. This increase can be to the effect of the polymer alone. Also, the reason for the change may be due to the nature of the wax because it contains a large amount of its composition and then the crystallus. Thus, have a soft texture may be avoided by vorroa while the growth of varroa in the control treatment. Hajij *et al.* 2014 agree with that, which stated that the application of ants acid led to a decrease in the total natural fall averages for the treatment group from 84.1 to 45.5 parasites/ day with a decrease of 45.9%. However, the average of natural drop of the comparison group increased from 49.7 parasites/day to 83.1 parasites/day and an increase of 67.3%. This reflects the positive effect of the use of ants acid in reducing the development of the varroa. A new application of anthrax was carried out using plastic containers. Hajij and al-Baraki (2006) reported that smoking with anise seeds gave the highest percentage of kills with smoking leaves, and mermia flowers, while the effect of the extract of water and alcohol of the black seeds in varroacontrol was weak and showed no effect on the bees. However, black seeds oil gave a good influence on vorroa and treat methods with natural plant extracts showed that there is no negative impact on bees (Alhajij, 2009).

Similar work confirm by Alberto (2005) which was on varroa and the effectiveness of an ant-acid starting from 29.6% and may exceed 90%. The effectiveness of an ant-acid using an anthrax gel, which called Bee Var formulation, or by Liebig-Dispenser ants acid between 93.6% and without any side disturbances in the hive.

**TABLE 2**: Number of varroa in hives before and after field experiments

| | Average of varroa before treatment | | Average of varroa after treatment | |
|---|---|---|---|---|
| Treatments | Brood | Adults | Brood | Adults |
| thymus oil polymer | 18.3 | 28 | 1 | 1 |
| Polymer only | 12.7 | 25 | 0 | 1 |
| Control | 12 | 16.3 | 10.33 | 7.67 |
| LSD | N.S | N.S | 5.328 | 1.762 |

The results for table 2 showed that there are no significant differences between treatments. The polymer alone treatment gave the lowest number of larvae into brood at the end of the experiment (0) and the rate of varroa on adults was (1) varroa. This treatment showed not different with thymus oil polymer, where the infection rates for brood and adults (1). However, in control treatment were (10.33, 7.67) respectively. This may be due to the action of the bio-polymer or the basic polymer components, where the polymer is characterized by bio-controlled system of effective oil. It is depending on the temperature degree.
This is confirmed with William *et al.* (2008) Vartiainen (*et al*., 2014) which they said that the goal of the biopolymer is the controlled release and starch of the starch and the amount of release necessary for control such as repellents, germicides, herbs, snails, and viruses as needed and antioxidant Spivak (1998) recorded that the varroa treated with thymus oil did not increase, which explains a decrease in varroa behaviour due to resistance genes. Zarog and ElBassiouny (2013), used thymol, garlic and on brood and they recorded that, it was effective High against the varroa parasite where the killing 61.7, 75.03 and 59%, respectively. Also, Mayltza *et al*., (2007) said that thymol removed 95% of avrroa and thymolwas consistently stable compared to other oils (Mikityuk and Grobov, 1979).Yin Chiesa (1991) used thymol against varroa for two seasons and they gave a good results of killing 96,77 and killing rate increased its effectiveness by adding sugar based on temperature.

**REFERENCES**

Alberto, S., Ignazio, F., Martin, E., Paolo, C., Luigi, G.V. and Marinella, M. (2005) Formic Acid-Based Treatments for Control of *Varroa destructor* in a Mediterranean Area. Journal of Economic Entomology, 98: 267-273

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.

Al-Sayegh, MuzahimAyoub, Faiz Abdul Shahad Abdul Hussain, Hussam Al-Din ThunounYounis and Abdul-Ahad Jabbo Mati (1990) Determination of the proportion of bees infected with the *Varroa jacobsoni* in the Nimrod region, conductively and chemically controlled during the autumn season, the second scientific conference of the University of Salahaddin.

Al-Zarog, A.A. and El-Bassiouny, A.M. (2013) Influence of some plant extracts on Varroa mite and performance of honey bee *Apis mellifera* colonies. Egyptian Academic Journal of Biological Sciences, 5 (2): 15–20.

ASTM D523 (1999) Annual Book of ASTM, American Society for Testing and Materials, Philadelphia, PA.

Baxter, J., Eischen, F., Pettis, J., Wilson, W.T. and Shimannki, H. (1998) Detection of fluvalinate resistant Varroa mites in US honey bees. Amer. Bee J. 138: 291.

Bryden, J., Gill, R.J., Mitton, R.A.A., Raine, N.E. & Jansen, V.A.A. (2013) Chronic sublethal stress causes bee colony failure. Ecology Letters, 16, 1463–1469.

CalisJ N.M., Boot, W.J. and Beetsma, J. (1999) Model evaluation of methods for *Varroa jacobsoni* mits control based on trapping in honey bee brood, A pidologie 30:197-207.

Ceylan, Hussein Sakr, Marza Hamza Hadi, Abbas Ghanem Hamza (2012) Effect of water and alcohol extract of the black seeds in the control the varroaon honey bees *Apis mellifera*, University of Baghdad, Journal of the Euphrates Agricultural Sciences, ISSN, Page- 114-118.

Chiesa, F., Agaro, M.D. (1991) Effective control of varroatosis using powdered thymol Apidologie,Springer Verlag, 1991, 22 (2), pp.135-145.

Chiesa, F., Agaro, M.D. (1991) Effective control of varroatosis using powdered thymol Apidologie, Springer Verlag, 1991, 22 (2), pp.135-145.

Fabra, M.J., Talens, P. and Chiralt, A. (2009) Food Hydrocolloids 23 676–683.

Grobov¸ O.F. (1976) Varroa in bees, Harnag¸ V. (Varroa honey bee disease) P .48-70 Apomondia. puplishing House Bucharest 1977.(Cited by AlMashadany¸M.¸1997).

Hajjeej, Nour al - Din Yusuf Dhahro Ali Khalid Al - Baraki (2006) Variety Department of Plant Protection, Department of Plant Protection Research, General Commission for Scientific Agricultural Research, Damascus, Syria, 9th Arab Conference of Plant Protection, 19-23 / 11/2006, Damascus, Syria. 66.

Hajjeej, Nour al-Din Yousef Zahero Ali Khalid Al-Baraki and Tamam Al-Abed (2014) Use of ant antidotes in the control of parasites on honey bees, Arab Plant Protection Code 32 (1): 57-63.

Hajjeej, Nour al-Din Youssef Zahero Ali Khalid Al-Baraki (2015) Effect of the division of honey bee colonies

*Apismellifera* in the development of the Varoa Jacbsoni community, Arab Plant Protection Program 33 (1): 55-59.

Hajjeej, Noureddine Taher (2003) Varroa control, Varroa jacobsonioud., Master Thesis in Agricultural Engineering (Plant Protection), Faculty of Agriculture, Damascus University, Syria, 127 pp.

Haubruge, E., Nguyen, B.K., Widart, J., Thome, J., Fickers, P., Depauw, E. Le depe rissement de and abeille domestique (2006) *Apis mellifera* L., 1758 (Hymenoptera: Apidae): faits et causes probables. Notes Fauniques Gembloux, 59, 3−21.

Henry, M., Be ´guin, M., Requier, F., Rollin, O., Odoux, J-F., Aupinel, P., Aptel, J., Tchamitchian, S., Decourtye, A. (2012) A common pesticide decreases foraging success and survival in honey bees. Science 336, 348–350. (doi: 10.1126 / science .1215039)

Katherine, J., Varpoucke, J., Delahitte Ritter, W. (2012) Varroa Tolerance in France of inter missa Bees From Tunisia and their Naturally mated des cendants, Bee. J. 144(7):563-568.

Langer, R. (1990) Polymer methods of drug delivery. Sci. 249:1527-1532.

Liebig, G. (1995) Kurzfassung desVortrages auf dem Apisticus-Tag am 21 Januar in Münster, Dtsch. Bienen J. 4-7.

Lozhechnikova, A., Dax, D., Vartiainen, J., Willför, S., Xu, C. and Österberg, M. (2014) Modification of Nanofibrillated Cellulose Using Amphiphilic Block-Structured Galacto glucomannans, Carbohydrate Polymers,110,163172.http://dx.doi.org/ 10.1016/j. carbpol. 2014.03 087.

Marinelli E., Formato, G., Vari, G. , De Pace, F.M. (2006) Varroa Control Using Celluose Strips Soaked In Oxalic Acid Water Solution Apiacta, 41: P. 54-59 54.

May-Itza, W.J., Medina, L.A. and Marrufo Olivares, J.C. (2007) Effectiveness of thymol based jells for the control of *Varroa destructor* mite that infests *Apis mellifera* honey bee colonies, under tropical conditions in Yucatan, Mexico. Vet.Mex., 38(1), 1-8

Mikityuk, V.V., Grobov, O.F., Chigareva, O.I. (1979) Trials of chemicals for controlling *Varroa jacobsoni* infestations of bees. Tr Vses Inst Exsf Vet 50, 120-125 (in Russian) (Apic Abstr 1982, 33, p 123)

Rand Esther E. du, Salome Smit, Mervyn Beukes, ZenoApostolides, Christian W.W. Pirk and Susan W. (2015) Nicolson. Detoxification mechanisms of honey bees *(Apis mellifera)* resulting in tolerance of dietarynicotine Scientific Repo Rts. 5:11779, DOi: 10.1038

Sahuki, Karima Abdel Wahib (1990) Applications in the design and analysis of experiments. El Hekmahouse For Printing & Publishing. University of Baghdad - Iraq.488 pages.

Smith¸ A.H. (2008) Pest management strategic plan for honey bees in the mid Atlantic states ¸Southern regional IPM center Virginia TECH¸ North Carolina State University.

Spivak, M., Gilliam, M. (1998) Hygienic behaviour of honey bees and its application for control of brood diseases and Varroa: Part II. Studies on hygienic behavior since the Rothenbuhler era. Bee World 79:169 –186.

William, McKee; doanesteven, W. and doane Milan, H-Savlch (2008) Methodes Of Making And Using Super absobent Polymers Product Including A Bioactive, Growth-Promoting Additive United States Patent (10) P616111 N0.I US 7,423,090 B2