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EFFECT OF SOME ALGAL METABOLITES PRODUCEDES FOR CONTROLLING *VARROA* MITE INFESTING HONEYBEE COLONIES

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Abstract: *Varroa*-infested honeybee colonies were exposed 5 times at 4 – day intervals to *Chroococcus minutus* (Kutz.), *Calothrix parietina* (Nageli.)., *Gloeocapsa giganteus* (Nageli.) and oxalic acid.

During February 2006, treated colonies were closed for about ¼ hour, then fallen *Varroa* mites which received on Vaseline-smeared plastic board installed on the bottom board were counted at the end of each application. Also, the effect of such treatments on brood rearing activity and spring honey crop were studied. Results are summarized as follows:

1- The number of captured *Varroa* in the treated colonies decreased gradually until it recorded the lowest values after the 5^{th} application. On the contrary, the inverse was true in case of control colonies.

2- Percentages of *Varroa* infestation decreased from 12.50, 13.50, 12.25 and 12.95% at the beginning of the experiment to 2.50, 2.13, 3.50 and 2.90% at the end of the treatment with ***Chroococcus minutus*** (Kutz.), ***Calothrix parietina*** (Nageli.), ***Gloeocapsa giganteus*** (Nageli.) and oxalic acid. While it recorded 12.15 and 26.26% at the start and the end of the experiment in untreated colonies.

3- The total sealed brood areas increased significantly in treated colonies with ***Chroococcus minutus*** (Kutz.), ***Calothrix parietina*** (Nageli.), ***Gloeocapsa giganteus*** (Nageli.) and oxalic acid, recording 927.20, 726.50, 886.10 and 853.60 $inch^2$ / colony, respectively compared to 582.80 $inch^2$ / colony in control. The respective percentages of increase of sealed brood areas basing in control colonies as 100% were 159.09, 124.66, 152.04 and 146.47%.

4- Exposing bee colonies to the *Chroococcus minutus* (Kutz.), *Calothrix parietina* (Nageli.)., *Gloeocapsa giganteus* (Nageli.) and oxalic acid were giving significant increasing in yield of citrus honey, recording 3.20, 2.21, 2.06 and 1.60 kg / colony respectively compared to 1.28 kg / control colony.

Key words: *algal metabolites, varroa* mite, honeybee.

Introduction

The ectoparasitic mite, ***Varroa Jacobsoni*** oud has become the most dangerous destructive pest for honeybees colonies since its invasion to Egypt, in 1987 up now (Yousif-Khalilt., 1992).

Under severe infestation, thousands of bee colonies would be completely destroyed. Such event had been taken place in Egypt during 1990 season. Even light or moderate infestation induce great damage to both of workers and drones, that represented by malformed wings, reduced body weight, shortened life span, reduced ability of flying to collect nectar and pollen, decreased development of hypo pharyngeal glands and reproductive organs and sperm production (Schneider *et al*., 1998; Kater, 1992; Yousif-Kalil, 1992).

The chemical control by Apistan, Folbex, Spinecar, Perizin, Malathion and Apitol is know to induce chemical pollution contaminating bee products (Mobus and Connor, 1988).

The present investigation aimed to study the control of ***Varroa mite*** infesting honeybee colonies by using cyan bacterial metabolites *(Chroococcus minutus* (Kutz.), *Calothrix parietina* (Nageli.), *Gloeocapsa giganteus* (Nageli.) and oxalic acid as a natural product.

The following parameters are studied:-

(1) Effect of products on the number of captured *Varroa* .

(2) Effect on the rate of infestation with ***Varroa jacobsini***.

(3) Effect on sealed brood area.

(4) Effect on citrus honey production.

In this concern, (Ibraheem and Nashaat, 2002) studied the toxicity effect of extra-cellular metabolites for three species *Gloeocapsa limneticus*, *Gloeocapsa giganteus* and *Phormidium tenue* against the two-spotted spider mite *tetranychus urticae Koch*.

Number of researchers were studying the replacement of chemical pesticides by natural components of different plant sources as insecticide agents; Pringsheim, (1949); Fogg, (1952); Hughes, *et al*., (1958); EL-Defrawi, *et al*., (1965); Allen, (1968); El-Ayouty, *et al*., (1976); Van-Der and Eloff (1983); Lustigman, 1988; Millamena, *et al*., (1990) Noda, *et al*., (1990) Frankmolle, *et al*., (1992) ; Nassar, *et al*.,(1995) ;Iskkander, *et al*., (1996); (Nassar, *et al*., (1999) and Amer, *et al*., (2000).

Material and Methods

The present investigations were carried out during spring season 2006 at Aboteg region, Assuit Governorate. Twenty honeybee colonies were similar in strength and rate of infestation with ***Varroa jacobsini***; were used in the present investigation; Colonies were headed by open mated

Carnio- Egyptian sister queens reared during July, 2005.

Cyanobacterial isolates:

The cultures were kindly obtained by Prof. Dr. I.B. Ibraheem. Botany Department, Faculty of Science, Beni-Suef University, Egypt.

Three cyanobacterial species were isolated from different Egyptian localities. ***Gloeocapsa limneticus*** and ***G. gigantcus*** isolated from El-Wadi-Sannur and *Phormidium tenue* isolated from Beni-Suef Wastewater Station.

Purification of algal isolates were carried out primarily by repeated culturing and subculturing on medium after Allen (1968) modified from Hughes Gorham and Zehnder (1958), until obtaining on final a pure unialgal cultures, which were then identified according Prescott 1951 and 1954. In order to obtain bacteria-free cultures, several trials were undertaken by using uni-algal culture (Pringsheim, 1949). For this main the antimicrobial treatment technique was applied (Felfoldy and Zsuzsa, 1959). The pure isolates of algae were inoculated in 100 ml sterilized liquid, Allen's medium having the same components as solid media contained in 250 ml conical flasks. These cultures were left to grow under 27±2 ^{0}C and light intensity of 4000 Lux. through light-dark cycle of 6-8 hrs, during the growth time. At the end of the incubation, the algal mass was separated by centrifugation at 3000 rpm. for 10 min under aseptic conditions. The resulting filtrates were cold sterilized and then used in the biological investigation.

The selected colonies were grouped, at random into 5 groups every of 4 colonies (replicates). All groups were subjected to four treatments and control:

1- Group (A) colonies were treated with *Chroococcus minutus* (Kutz.), 4-6 pufos/comb

2- Group (B) colonies were treated with *Calothrix parietina* (Nageli.).

3- Group (C) colonies were treated with *Gloeocapsa giganteus* (Nageli.).

4- Group (D) colonies treated with oxalic acid.

Thereafter the hive entrance was tightly closed for about ¼ hour. A plastic board smeared with raw Vaseline was installed on the bottom board of the hive to act as a trap capturing the fallen mites after all treatments, a wire screen of nearly 10 mesh / inch was installed over the plastic board to prevent bees from touching the Vaseline layer. This procedure was repeated five times at 4-day intervals starting on Feb. Captured ***Varroa*** mites were counted and removed at the end of each interval.

5- Group (E) untreated colonies were as control.

In addition to the counting of captured *Varroa* mites every 4 days after all treatments, the following parameters were measured:

A – Brood rearing activity:

The sealed brood area was determined using standard frame divided into square niches every 12 days intervals starting on the first of March and lasted on April 6, 2006.

B – **Citrus honey production:**

Citrus honey produced by tested colonies were evaluated for each colony individually as a difference between the weight of honey combs before and after extraction (the weight was calculated in Kgs±10 gm).

C – Infestation rate:

The rate of ***Varroa*** infestation in tested colonies was determined twice, the first was carried out at the beginning of the experiments and before any treatment application, while the second was made at the end of the five treatments according to the following procedure:

Hundred workers were picked up randomly from each colony such workers were anesthetized with ether, and then placed in a glass funnel. An amount of about 200 ml. of hot water was then poured on them. A beaker was used to receive such water with the fallen ***Varroa*** mite. A piece of wire screen mesh was placed on the bottom of the funnel to prevent bees drift with hot water, there of number of mites found in the received water was recorded as the percentage of infestation.

Obtained results were statistically analyzed according to Snedecor (1957) methods.

Results and Discussion

1– Effect of treatments on the number of captured *Varroa* :

Data in Table (1) indicated that treating *Varroa* infested honeybee colonies with *Chroococcus minutus* (Kutz.), *Calothrix parietina* (Nageli.) ,*Gloeocapsa giganteus* (Nageli.) and oxalic acid.

For instance, the mean total numbers of captured ***Varroa*** were 1001.9, 1697.85, 1222.60 and 2305.00 mites/colony in groups A, B, C and D, respectively, compared 172.30 mites in control colonies. However, honeybee colonies of group (D) showed the highest number of fallen ***Varroa***, being significant as compared to that of groups A, B, C and E.

In this respect, effect of some plant materials in controlling ***varroa***, *jacobsini* infesting honeybee colonies were reported by several authors such as Sadov *(1981);* Marchetti *et al., (1984); EL-Santil (1990).;* Fathyand Fouly *(1997);* Imdorf, *et al. (1999);* and Hussien, *et al. (2001).* In addition, the number of captured *Varroa* was the highest after the first application in all treatments, then it decreased gradually until recording the lowest number of fallen *Varroa* after the last application indicating the efficiency

used of cyanobacterial metabolites (Table 2).

In this respect, effect of some plant materials in controlling *Varroa jacobsini* infesting honeybee colonies were reported by several authors such as Sadov (1981) ; Marchetti *et al*., (1984); EL-Santil (1990).; Fathyand Fouly (1997) ; Imdorf, *et al.* (1999); and Hussien, *et al.* (2001).

In addition, the number of captured *Varroa* was the highest after the first application in all treatments, then it decreased gradually until recording the lowest number of fallen *Varroa* after the last application indicating the efficiency used of cyanobacterial metabolites (Table2)

2– Effect on the percentage of infestation:

Data presented in Table (1) indicated clearly that the percentages of *Varroa* infestation, calculated as the number of mites / 100 workers, recorded the means of 12.50, 13.50, 12.25 and 12.15 in the colonies of groups A, B, C, D and control, respectively. The respective percentages of infestation at the end of the experiments were decreased significantly in bee colonies treated with *Chroococcus minutus* (Kutz.), *Calothrix parietina* (Nageli.), *Gloeocapsa giganteus* (Nageli.) and oxalic acid as compared to that of control colonies being 2.50, 2.13, 3.50, 2.90 and 26.26% (Table 1).

3 – Effect on sealed brood area:

Data presented in Table (1) showed that exposing honeybee colonies to *Chroococcus minutus* (Kutz.), *Calothrix parietina (*Nageli.), *Gloeocapsa giganteus* (Nageli.) and oxalic acid A, B, C, D 5 times at 4-day intervals during February affected significantly brood rearing activity. For instance, the mean total of sealed brood area measured after application during the period extending from February 26, to April 6, 2006, recording 927.20, 726.50, 886.10 and 853.60 inch^2 / colony for A,B,C and D colonies, respectively. The respective area for control colonies recorded 582.50 inch^2 / colony. However, the difference between treatment A and control was significant (Table 1).

In addition, it was noticed that sealed brood area increased gradually, in all treatments including control, starting from February 26, reaching the maximum area in March 24, recording 298.60, 240.30, 254.00, 156.30 and 196.40 (inch^2 / colony) for colonies treated with *Chroococcus minutus* (Kutz.), *Calothrix parietina* (Nageli.), *Gloeocapsa giganteus* (Nageli.) and oxalic acid and control, respectively (Table 3). Thereafter, a remarkable decrease in sealed brood area took place in all treatments during the next interval. This decrease could be attributed to decrease the egg laying capacity where it due to the highest nectar gathering and citrus honey storing activity. In this respect,

Khattab, (1967), respected that citrus flowering period in Egypt extends from the last week of March till mid – April.

4– The effect on citrus honey production:

Obtained results indicated that exposing bee colonies 5 times at 4 – day intervals during February *Chroococcus minutus* (Kutz.), *Calothrix parietina* (Nageli.), *Gloeocapsa giganteus* (Nageli.) and oxalic acid induced significantly citrus honey production during lat week of March and the first half of April by such colonies as compared to that of untreated (control) colonies. For instance, the mean honey production recorded 2.203, 2.205, 2.060 and 1.600 Kg. / colony for the experimental colonies in group A, B, C and D, respectively compared to 1.275 Kg. / colony for control ones (Table 1). It is obvious that the treated colonies with the four treatments yielded a highly significant greater quantity of honey crop as compared with control colonies. However, the differences in this parameter between the four treatments A, B, C and D, were insignificant. The increased quantity of crop honey in treated colonies could be attributed to many factors, such as the higher colony population, the lower percentage of *Varroa*-diseased workers and the higher brood rearing activity imitated in such colonies which induced more nectar and pollen collection (Free, 1978). In addition colonies diseased with *Varroa* have workers lower ability of flying to make collection tours and lower capacity of lodging nectar and pollen and shorter life span (Schneider *et al*., 1988; Mobus and Connor, 1988; Yousif – Khalil, 1992).

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تأثير بعض المنتجات الأيضية لبعض الطحالب في مكافحة حلم الفاروا الذي يصيب طوائف نحل العسل

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تمت معاملة طوائف نحل العسل المصابة بالفاروا بـ ***Chroococcus minutus*** (Kutz.), ***Calothrix parietina*** (Nageli.),***Gloeocapsa giganteus*** (Nageli.) and oxalic acidخمسة مرات بمعدل مرة كل أربع أيام خلال شهر فبراير 2006 0 وكانت الطوائف بعد كل معامل تقفل بإحكام لمدة ربع ساعة وتم استقبال الحلم المتساقط على لوحة بلاستيكية مغطاة بطبقة من الفازلين وموضوعة على قاعدة الخلية حيث تم عدة مرة واحدة في نهاية كل دورة علاج 0 بالإضافة إلى ذلك تم دراسة تأثير هذه المعاملات على نشاط الطوائف في تربية الحصنة ومحصول عسل الموالح لهذا الموسم ويمكن تلخيص النتائج كما يلي:

1ـ تناقص عدد الفاروا المتساقط تدريجياً مع تكرار المعاملة حتى سجل أقل قيمة بعد المعاملة الخامسه و العكس صحيحاً مع طوائف المقارنة 0

2ـ تناقص النسبه المئوية للأصابة بالفاروا من 12.50 ، 13.50 ، 12.25 ، 12.95% في بداية التجربة إلى 2.50 ، 2.13 ، 3.50 ، 2.90% عند نهاية التجربة وذلك نتيجة المعاملة بكل من ***Chroococcus minutus*** (Kutz.), ***Calothrix parietina*** (Nageli.), ***Gloeocapsa giganteus*** (Nageli.) and oxalic acid بينما زادت هذه النسبة من 12.15% إلى 26.26% على الترتيب عند نهاية التجربة في طوائف المقارنة 0

3ـ أدت المعاملة بهذه المواد إلى زيادة معنوية في مساحة الحضنة المقفلة في الطوائف مسجله مجموعاً قدره 927.20 ، 726.50 ، 886.10 ، 853.60 بوصة مربعة / طائفة معاملة بـ ***Chroococcus minutus*** (Kutz.), ***Calothrix parietina*** (Nageli.), ***Gloeocapsa giganteus*** (Nageli.) and oxalic acid وذلك مقارنة بـ 582.80 بوصة مربعة حضنة مقفلة / طائفة على الترتيب فى المقارنه, وبلغت النسبة المقارنة للزيادة في مساحة الحضنة المقفلة في المعاملة 159.09 ، 124.66 ، 152.04 ، 146.47% بنفس الترتيب على اعتبار أن الحضنة في طوائف المقارنة قد سجلت 100%0

4ـ أدت معاملة طوائف نحل العسل بـ

Chroococcus minutus (Kutz.), ***Calothrix parietina*** (Nageli.), ***Gloeocapsa giganteus*** (Nageli.) and oxalic acid إلى حدوث زيادة معنوية في محصول عسل الموالح والذي سجل 3.20 ، 2.21 ، 2.06 ، 1.60 كجم عسل / طائفة على الترتيب وذلك عند مقارنته بـ 1.28 كجم عسل / طائفة مقارنة 0