EFFECT OF SOME ANTIBIOTICS ON BROOD REARING ACTIVITY IN HONEY BEE COLONIES AND THE MICROFLORA IN THE PRODUCED HONEY

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Summary

Fifteen colonies of hybrid carniolan bees of equal strength were chosen from the apiary of the Agriculture Faculty at Mansoura, Egypt, in 1995, for carrying out the experiments. The influence of four antibiotics, i.e. flumox, flumox + V. B12, velosef and velosef + V.B12 on brood rearing activity and on the microflora in produced honey during bee feeding with sugar-water solution (v/v) was studied.

The results indicated that the colonies treated with flumox + V.B12 were more active on brood rearing activity (2552 sq. in., average of total number of brood sq. in.) and produced more honey (129.4%, average 5.5 kg/colony) than any other treated with other antibiotics or untreated. The colonies treated with velosef alone the lowest colonies in brood rearing activity (1038.9 sq. inch, average of brood area/colony) and honey production (72.9%, average 3.1 kg/colony).

The results showed also that the microflora (as cfu/ml) in honey from colonies treated with antibiotics were less aboundant than that from colonies with antibiotics added vitamin (V.B12).

Key words: bee colony, antibiotic, brood rearing, honey, microflora.

Introduction

The advent of antibiotics marked in a few years an even greater advance in chemotherapy. This group of therapeutic agents has been aptly defined by WAKSMAN (1956) as chemical substances, that produced by microorganisms have the capacity of inhibit and even destroy microorganisms.

Many trials were made by entomological investigators to use antibiotics for the control of the diseases of some useful insects such as honey bee (*Apis mellifera* L.). Some of these investigations were made on honey bee activity and development of colonies, such as VOVK (1963) who during examination of the twelve colonies dusted with biomycin, found that the experimental colonies reared more brood (51.5%) and produced more honey (20.8%) than those of the control group.

ROBERT (1965) recorded that using some antibiotics at least one month before the major honey flows, there is no danger of residues appearing in the honey.

SMIRNOV (1969) suggested that commercial honey obtained from apiaries treated by antibiotics should be kept for a suitable period before sale so that the antibiotics in tem will lose their activity.

The honey-bee, which fly long distances to collect nectar and pollen are subject to contamination with various types of microorganisms from different sources. EL-LEITHY and EL-SIBAEI (1972) isolated bacteria and yeasts from the surface and internal parts of health bees. From another point of view honey, though considered to be relatively stable foodstuff, is susceptible to fermentation by certain microorganisms (STEPHEN, 1946 and MARTIN, 1958). These may be incorporated in honey by the bees or many come from contaminated utensils.

The present work aim was to study the effect of the addition of some antibiotics to the honey bee food on brood rearing activity, and the microbial content in the produced honey.

Materials and Methods

A) Testing of Antibiotics on Brood Rearing

Fifteen honey bee colonies of hybrid carniolan bees of equal strength headed by sister queens were chosen in April 1995 from the apiary of Agriculture Faculty at Mansoura, for carrying out the experiments. The five used treatments were as following and each treatment was replicated on 3 colonies:

<u>Group A:</u> - provided with food only (used as control);

Group B: - treated with flumox (its structure as following):







Group E: - treated with velosef (cephardine) + V.B12

The antibiotics was provided to the experimental colonies of honey bee (*Apis mellifera* L.) at 0.015% constituent in a 2:1 sugar-water solution (v/v). Each colony was fed once a week, each time with 500 g of the sugar-antibiotic mixture.

Areas containing sealed brood were measured at intervals of 12 days in square inches (sq. in.) according to FRESNAY and LENSKY (1961).

B) Microbiological Examinations

<u>Enumeration</u>: Serial decimal dilutions of five samples of the produced honey from colonies either treated or untreated were prepared. Plate count technique was used for viable cell counts using basal medium (beef extract, 0.3%; yeast extract, 0.2%; tryptone, 0.5%; glucose, 1%; K_2HPO_4 , 0.2%; agar, 1.5%; in distilled water, pH 7). Triplicate plates were prepared for each dilution and incubated at 30 ^oC for appropriate time (1-7 days). Well separated colonies were estimated and related to one ml honey (as cfu/ml).

<u>Isolation and Purification:</u> Well separated colonies, from the plates for colony counts on the forenamed medium, were retransferred to agar slants. All isolates were purified by streaking on appropriate agar plates. Cultures were considered pure when they showed uniform morphological features after 24 hours incubation on nutrient agar for bacteria, 48 h for yeast and 7 days incubation for mould on potato dextrose agar (PDA, Merck).

<u>Characterization of the isolated strains</u>: By microscopical examination *Cladosporium* sp. was identified according to ELLIS (1970). For differentiation of yeasts the scheme of KREGER-VAN RIJ (1984) was followed. The bacteria were grouped according to cell morphology and gram reaction, all the types were determined according to BERGEY's Manual (1984).

Results and Discussion

A) Effect of Antibiotics on Brood in the Honey Bee Colonies

With respect to brood rearing activity, the obtained results were recorded in Table 1 and Figs. 1 and 2. The average of total number of worker sealed brood areas (square inches per colony) were 2552, 1760.6, 1743.6, 1595.8 and 1038.9 for each treatment, respectively, as shown in Table 1.

| Rate of brood rearing activity in colonies treated with different antibiotics | | | | | | | |
|---|--|---------------|---------|--------|---------|--|--|
| Date | Average of sealed brood area (sq. inch/colony) | | | | | | |
| | Flumox + V.B12 | Velosef+V.B12 | Control | Flumox | Velosef | | |
| 18/4/1995 | 110.1 | 116.5 | 85.5 | 125.1 | 62.1 | | |
| 30/4/1995 | 168.8 | 90.5 | 84.1 | 167.8 | 68.5 | | |
| 12/5/1995 | 300.5 | 226.8 | 181.5 | 147.8 | 72.2 | | |
| 24/5/1995 | 451.5 | 364.1 | 281.5 | 256.1 | 180.5 | | |
| 05/6/1995 | 494.8 | 334.5 | 389.5 | 310.8 | 205.1 | | |
| 08/7/1995 | 463.5 | 302.5 | 356.1 | 178.8 | 134.5 | | |
| 20/7/1995 | 252.8 | 119.1 | 103.1 | 151.1 | 103.8 | | |
| 01/8/1995 | 134.5 | 84.5 | 119.1 | 139.8 | 90.1 | | |
| 13/8/1995 | 175.5 | 122.1 | 143.2 | 118.5 | 122.1 | | |
| | 2552 | 1760.6 | 1743.6 | 1595.8 | 1038.9 | | |

ate of brood rearing activity in colonies treated with different antibiotics

Table 1

The maximum average number of worker sealed brood area (sq. inches/colony) was estimated on the first week of June when it reached 494.8 sq. inches in the colonies treated with flumox + V.B12, while in the control colonies it was 389.5 sq. inches.

The results illustrated in Fig. 1 were similar to the previous data which appeared that treated colonies with flumox + V.B12 gave the highest number of brood sq. in. (2552) over a longer period than any other treated compound, while velosef (1038.9 sq. in.) was the lowest compound.

Figure 2 illustrated the comparison between the four used compounds and the control using average number of worker brood sq. inches during the experimental period.

Comparing the treated colonies with the four compounds of antibiotics and the honey production, it was considered that untreated colonies gave 100% honey production (av. 4.25 kg/colony), flumox + V.B12 129.4% (av. 5.5 kg/colony), velosef + V.B12 94.1% (av. 4.0 kg/colony), flumox 88.2% (av. 3.75 kg/colony) and velosef 72.9% (av. 3.1 kg/colony).

It could be mentioned that the brood rearing activity under the influence of those antibiotics greatly affects honey production in a similar way.

Data concerning brood rearing activity as indicated in Tables 1 and 2, revealed that the colonies treated by flumox + V.B12 were significantly more active in brood rearing than those treated by velosef + V.B12, flumox, velosef and untreated colonies. Furthermore, the results generally showed that brood rearing activity was significantly less in velosef than any other treatment.

| L.S.D. test between the four coumpounds | | | | | | | | |
|---|----------------|-----------------|---------|--------|---------|--|--|--|
| Antibiotic Comp. | Flumox + V.B12 | Velosef + V.B12 | Control | Flumox | Velosef | | | |
| Av. sq. in brood | 283.6 | 195.6 | 193.7 | 177.3 | 115.4 | | | |
| Significancy | а | b | b | С | d | | | |
| | | | | | | | | |

Similar initial indicates an insignificant difference

In Table 3, it was evident from the results and observations that the average number of brood rearing sq. in. reached its maximum (346.94 sq. in.) during the honey flow at the 5th of June.

Table 3

Table 2

L.S.D. test between the dates after treatment with antibiotics

| Date | Average of sealed brood area (sq. inch) | Significancy |
|-----------|---|--------------|
| 18/4/1995 | 99.86 | С |
| 30/4/1995 | 115.94 | а |
| 12/5/1995 | 185.76 | d |
| 24/5/1995 | 306.74 | е |
| 05/6/1995 | 346.94 | f |
| 08/7/1995 | 287.08 | g |
| 20/7/1995 | 160.62 | h |
| 01/8/1995 | 113.60 | а |
| 13/8/1995 | 136.28 | b |

Similar initial indicate an insignificant difference.



Fig. 1 – Brood rearing activity of treated colonies antibiotics (------) compared with the untreated (--) colonies during the active season, 1995



Fig. 2 - Total counts of sealed brood area (sq. inch/colony) under the effect of antibiotics during the active season, 1995

Concerning the effect of feeding colonies with antibiotics on healthy colonies, the previous results indicated that colonies treated with flumox + V.B12 were more active in brood rearing and produced more honey than any other colonies treated with other antibiotics, or untreated colonies. These results are supported by KAPANEVICH (1963, 1964) who noticed an evidence that antibiotics have a stimulating effect on healthy bees, increase in appetite, acceleration of development of the strength of the colonies, and resulting in an increased honey production. He added that healthy colonies receiving antibiotics produced more honey, had a better production, lived longer, more active flow out in cold weather, even in drizzle, and finished their daily flight activity later than the control.

Data indicated that, colonies treated with flumox alone were less in brood rearing activity and honey production that any other treatment, while colonies treated with velosef alone were the lowest colonies in brood rearing activity and honey production.

B. Effect of Antibiotics on the Produced Honey Microflora

Actually a heterogeneous mixture of micro-organisms including bacteria, yeasts and molds were found in the produced honey, total count reached to 2×10^4 cfu/ml (Fig. 3). This microflora was reduced about 25% in the produced honey from colonies treated with antibiotics (either velosef or flumox). But the reduction was less in the produced honey from colonies treated with antibiotics + vitamin (B12).



Fig. 3 – Microbial content of produced honey from treated colonies with antibiotic or antibiotic + vitamin

The most frequent micro-organisms in the produced honey samples (either treated or untreated) were isolated. The isolates involved moulds (belong to the genus *Cladosporium*), yeasts (belong to the genus *Saccharomyces*) and bacteria (the predominant aerobic spore forming bacilli belong to *Bacillus subtillis*, *B. megatherium* and *B. polymyxa*, in addition were micrococci belonging to *Micrococcus luteus* and *Micrococcus varians*). Some of these microorganisms seem to have been recognized in and on honey-bee (EL-LEITHY and EL-SIABEI, 1972). Under conditions of honey ripening, these microorganisms of their enzymes are translocated with the honey and may have an important role in the process of formation of honey from nectar (MARTIN, 1958). It could be expected that the addition of antibiotics to the colonies influences the composition of the microbial flora in the produced honey.

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