

## Effect of Some Antimicrobial Drugs on the Fitness of Honeybee *Apis mellifera* L. (Hymenoptera: Apidae)

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### ABSTRACT

Antimicrobial drugs are used by beekeepers to control bacterial and microsporidian infections. This raises concerns among individuals all over the world. The current work aims to determine the residue concentrations of three commonly used antibiotics (tylosin, sodium sulfademedin, and septazol) used to control honeybee infections, as well as to investigate the effects of antibiotics on bee biology. Honey was collected 42 days (2 generations of the worker development) post-treatment for HPLC analysis; honeybee activities (brood-rearing and pollen areas) were evaluated at 5 intervals every 12 days (0, 12, 24, 36, and 48 days) post-treatment. Hygienic behavior and metabolic reserves (body proteins, lipids, and carbohydrates) were assessed at two intervals (0- and 42-day) post-treatment. Results showed that residues of antimicrobial drugs in honey samples were significantly higher than those in the controls. Results indicated that no negative effect was observed on the brood rearing and pollen gathering as well as hygienic behavior of honeybee workers due to the doses of the antibiotics. Meanwhile, the colonies treated with tylosin showed lower hygienic behavior than the control at 0-day. The time progress did not affect the brood-rearing area in all tested conditions. The metabolic reserves showed non-significant differences post-treatment compared with the controls. However, the body proteins and lipids exhibited lower contents at 42 days compared to those at 0-day. This study found a significant contamination risk for bee products as a result of inadequate apiculture techniques, as well as a possible risk to honeybees.

**Keywords:** Antibiotics, Bee activity; Honeybee fitness; Metabolic reserves.

### INTRODUCTION

The honeybee *Apis mellifera* L. (Hymenoptera: Apidae) is the most important pollinator. Its colonies are globally used for the pollination of many crops and fruit grown in the open fields as well as numerous wildflowers (Klein *et al.*, 2007, Blaauw and Isaacs, 2014). In addition, bee products are widely used as food, pharmaceutical drugs, and cosmetics (Zbucnea, 2017). Many interacting factors such as poor nutrition, parasites, pesticides, antibiotic exposure, climate changes, and habitat loss could cause colony decline (Alaux *et al.*, 2010, Potts *et al.*, 2016). Colony collapse disorder (CCD) was first reported in 2006 when the adult honeybees disappeared from the hives leaving behind a queen and a few nurse bees (Lee *et al.*, 2015).

Sulfonamides and antibiotics are worldwide used in beekeeping to prevent or control honeybee diseases (Hansen and Brødsgaard, 1999, Williams, 2000). However, the abuse of these antimicrobials could leave residues in the beehive products. Some researchers found sulfonamides and tylosin residues in the honey (Zhang *et al.*, 2019). Abd Alla (2020) also recorded several residues in the honey sample collected from different regions in Egypt. Antibiotic residues show a long half-life and they may have toxic effects on consumers or cause allergic reactions in hypersensitive individuals (Tillotson *et al.*, 2006). The council regulation (EEC, 1990) and Egyptian Honey Specifications Standard (EOS, 2005) stated that the bee

honey must not contain any antibiotic residues, i.e. no maximum residues limits (MRLs). So that, it is desired to screen the presence of residues in bee honey after antimicrobial treatment by the beekeepers.

Hygienic behavior (HB) is an example of the social behavior of honey bees and a natural mechanism for the control of disease by removing dead brood (Lapidge *et al.*, 2002). HB is an important behavior against the American and European foulbrood, and chalkbrood (Gilliam *et al.*, 1983) and resistant mite, *Varroa destructor*, by removal infected pupae (Spivak, 1996). Some studies determined the level of HB for *A. mellifera acarnica* and *A. mellifera jemenitica* (Kamel *et al.*, 2003, Balhareth *et al.*, 2012). Woyke *et al.* (2012) observed that almost 89% of the freeze-killed brood was removed by the bees within 24 h while 95% of the pin-killed brood was removed within 48 h after introduction. However, HB was observed to decrease on exposure to oxalic acid (Bacandritsos *et al.*, 2007).

Pollen quantity affects bees' physiology and immune competence (Field *et al.*, 2002, Di Pasquale *et al.*, 2013). Some studies observed a relation between the pollen area stored and the brood-rearing area (Standifer, 1980). Shortage of pollen area and brood-rearing area affect the vigor of the colony and may cause colony decline or collapse (Neupane and Thapa, 2005). Fat bodies in bees, like the liver in humans, are responsible for energetic metabolism storage and conversion of protein, fat, and carbohydrates (Arrese and Soulages 2010). Abd El-Nabi *et al.* (2014) reported



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a high increase in the total proteins content and total carbohydrate with a diet supplement. Treatment of honeybee colonies with antibacterial such as Marjoram oil, oxalic acid, and tylosin caused a reduction of the worker hemolymph protein levels (Nafea *et al.*, 2013). Other studies demonstrated a decrease in hemolymph protein, lipids, and carbohydrates after treatment with Bayvarol and Apivar (Bogaerts *et al.*, 2009). Sometimes, these antibacterials may cause drops in carbohydrates and fat, while increasing proteins in the hemolymph after treatment with oxalic acid (Adjlane *et al.*, 2013). In the current study, the presence of antimicrobial residues was screened in the bee honey following the common treatment used by the beekeepers, and the effects of these antimicrobials were evaluated on honeybee biology and physiology.

## MATERIALS AND METHODS

### Honeybee and treatments

This study was carried out at the apiary yard of the Bee Research Department, Plant Protection Research Institute, Dokki, Giza Governorate, Egypt from 18 June to 15 August 2020. The average density of bees per hive was approximately equal (at least seven combs covered with bees and headed by new mated carniolan hybrid queens). Twelve healthy hybrid carniolan honeybee colonies, *Apis mellifera* were divided into four groups: three treatments and one control; each with three replicates (colonies). Three antimicrobial drugs, tylosin, sodium sulfademedin, and septazol were used as well as the control. Control colonies were fed with (1:1) volume sugar solution.

### Antimicrobial Dosage

Tylan® (tylosin tartrate; El Nassr Pharmaceuticals Chemicals Co, Cairo, Egypt) was applied as a suspension, which was prepared by dissolving 0.25 g tylosin in 500 ml sugar solution (1:1) according to the Reybroeck method (2002). Suspension of sodium sulfademedin (sulfamethazin; Alexandria Pharmaceuticals & Chemicals Industries Co, Alexandria, Egypt) was prepared by dispersing 0.75g sodium sulfademedin in 500 ml sugar solution (1:1). Sulfamethooxazol, trimethoprim; Alexandria Pharmaceuticals & Chemicals Industries Co, Alexandria, Egypt) was used as a suspension by mixing 1 mL septazol with 500 mL sugar solution (1:1). These solutions were applied as food every four days/four times (Zidan, 2014). By the end of the application, this was set as 0-day. Controls were supplied with an equal amount of the same amount of food sugar solution (1:1) devoid of antimicrobial.

Samples were collected from all honey-bee colonies (honey and bee workers) at 0- and 42-day (2 generations of the worker development) after the end of the application (12 days). This time frame (42 days) is supposed to be sufficient to reduce the antibiotic content of the honey harvested. The experiments were conducted in 3-6 biological replicates according to the experimental conditions.

### Determination of residual antibiotics (LC/MSMS)

Honey samples were collected 42 days post-treatments for chemical analysis using Liquid Chromatography-Tandem Mass Spectrometry (LC/MSMS). Sulfamethazine and sulfamethooxazol were solubilized in methanol at a concentration of 0.5 mg/l and held at -20 °C until utilized, whereas tylosin standard was solubilized in water at a concentration of 1 mg/ml.

Pure sunflower honey was provided by an Egyptian beekeeper, which was evaluated and used as a blank honey. Honey samples (1.0 ±0.1 g) were dissolved in a 0.5 ml disodium hydrogen phosphate buffer solution (0.1 M, pH 8). Acetonitrile, the first extraction, was added to the sample and agitated for 10 minutes with a shaker (K-500, Janke & Kunkel, Breisgau, Germany), then sonicated for 2 minutes with an ultrasonic bath (Branson 2510, Branson Ultras-onics, Danbury, CT, USA). The mixture was centrifuged at 3632 *xg* (Mistral 2000, MSE Scientific Instrument, Crawley, UK) for 3 min at room temperature then collected into a new glass tube, dried under a stream of nitrogen at 45 °C and stored at room temperature till used. Extraction No. 2 hydrolyzed sulfonamides in a water bath at 65 °C for 1 h, and then cooled at 4 °C for a few min. The mixture was neutralized by adding 120 ml of 1 M disodium hydrogen phosphate (pH 12). One ml acetonitrile was added to the hydrolyzed sample, shaken for 10 min, and then sonicated, centrifuged, dried as above, and stored at room temperature till used.

The two dried extracts were individually suspended with 100 µL of methanol/water (20:80, v/v). After cautious vortexing and sonication, each honey extract was filtered through a 0.45 mm pored nylon filter (Nalgene, Rochester, NY, USA) directly into distinct HPLC vials and then stored at -20 °C till analysis. Analysis was performed on an Agilent 1100 pump LC system coupled to a 4000 Q TRAP mass spectrometer instrument equipped with a turboboion spray ionization source. HPLC analysis was run on azorbax SB-C18 reverse-phase column (2.1×50 mm, 1.8 µm). The mobile phase was constituted with solvent A (water containing 1 Mm NFA mixed with 0.5% formic acid (v/v) and solvent B (acetonitrile/methanol (50:50, v/v) containing 0.5% formic acid (v/v).

The gradient program was 0 min 0% B, 0-0.1 min 25% B, 0.1-3 min 25% B, 3-7 min 80% B, 7-12 min 95% B, 12-14 min 99% B, 14-15 min 0% B, 15-25 min 0% B; running at a flow rate of 0.3 ml/min the injection volume was 10 µl of each of the four honey extracts and was delivered into the High-performance liquid chromatography (HPLC) column. Quantitative analysis was performed using the most intense selected reaction monitoring (SRM) signal (SRM1) whereas the second SRM transition (SRM2) was used for analyte confirmation based on the appropriate area ratio calculated from the standard solution as described by Hammel *et al.* (2008). Data processing was performed using analyte 1:4:1 software Applied Biosystems MDS/SCIEX).

### Measurements of brood area and pollen area

The rearing areas occupied by unsealed and sealed worker brood and pollen area stored were measured using a frame divided into square inches on 0, 12, 24, 36, and 48 days post-treatment according to the method of Moosbeckhofer and Bretschko (1996). These measurements were performed in three biological replicates for each treatment along with the control.

### Hygienic behavior (HB) measurements

An area (4-inch square equal 100 cells) of sealed worker brood was killed by piercing a thin metal pin into each cell and then the comb was returned to the hive to record HB according to the method of Sammataro (1996). The percentage of brood removal in each colony was recorded 24 h after piercing. These measurements were done two times at 0- and 42-day following antimicrobial treatment as well as in the control colonies. For each treatment and the control, this calculation was carried out in three biological replicates. Hygienic behavior was calculated according to the following equation:

$$HB = \frac{(X - Y - Z)}{X} \times 100$$

Where X is the number of brood cells that were perforated and killed using a pin; Y, is the number of cells that remained capped, and Z is the number of uncapped cells with dead brood inside.

### Preparation of the honeybee body homogenate

One honeybee worker approximately ( $0.1 \pm 0.01$  g) weight was sterilized using 70% methanol solution and put in an Eppendorf tube with 0.5 ml phosphate-buffered saline, PBS (0.1M, pH 7.8). Each sample was homogenized using fastgene® mixy homogenizer (NIPPON Genetics EUROPE GmbH, Dueren, Germany) for 10 min on ice and centrifuged (3-30KS, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 1000 xg, 4 °C for 10 min. The supernatant is decanted into a new Eppendorf tube and kept till used at -80°C ultra-freezer (Daihan Scientific, Korea) (Abd El-Nabi *et al.*, 2014). This procedure was repeated 6 times (biological replicate) in each treatment and the control. Biochemical tests were conducted at the Department of Zoology, Faculty of Science, Tanta University.

### Determination of total protein, lipid, and carbohydrate contents

The total body proteins were determined in the samples according to the colorimetric method described by Henry (1964) using a kit (Diamond®, Egypt). The sample and standard (albumin) were measured against a blank at 550 nm on a spectrophotometer (UNICO S1200, USA). The lipid content was also determined using the method of Zöllner and Kirsch (1962) at 540 nm against olive oil as a standard according to the instructions of the kit manufacturer (Biodiagnostics®, Egypt).

The total carbohydrates were estimated according to the method described by Singh and Sinha (1977). The absorbance of samples and a series of strands glucose were measured at 620 nm. This procedure was repeated

6 times per treatment. The total carbohydrates content was estimated using the equation of the regression line:

$$Y = 0.096X + 0.0074.$$

Where Y is the absorbance at 620nm and X is carbohydrate concentration (g/dl).

### Statistical analysis

Data were expressed as mean  $\pm$  standard deviation (M $\pm$ SD). The effect of treatment with antibacterial drugs was investigated in the bee honey samples using an unpaired t-test. The level of significance was set on  $p \leq 0.05$ ,  $p \leq 0.01$  and  $p \leq 0.001$ . The effect of treatment on brood rearing and pollen areas was analyzed using regression with time elapsed. Two-way ANOVA was used to study the effect of treatment and antimicrobial type on HB and metabolic reserves. Multiple comparisons among the antimicrobials (Bonferroni) were used for in-depth analysis. These analyses have been done in GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, [www.graphpad.com](http://www.graphpad.com).

## RESULTS

### Residual concentration of antimicrobial drugs in bee honey samples

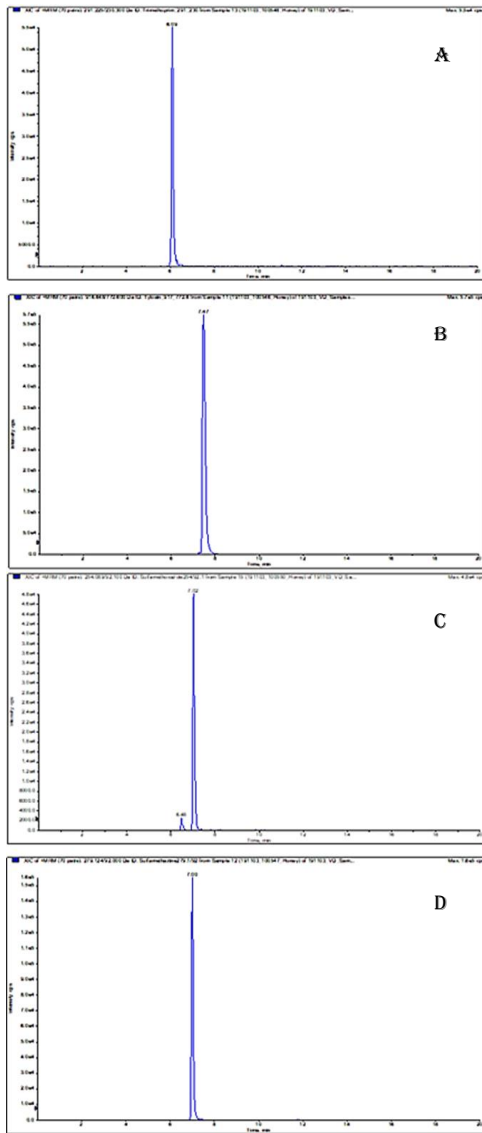
Antibiotic residues (Tylosin, sodium sulfademedin, and septazol) used in honeybee treatment were detected in the honey produced. Figure (1) demonstrates the detected antibiotic used when the harvested bee honey samples were analysed using (LC/MS) MS after 42 days after treatment. In comparison to the control honey bee samples, treated bees in colonies after 42 days showed a higher concentration of antimicrobial drugs (Table 1). According to the results of t-test, the honey samples contained considerably higher tylosin ( $t = 16.7$ ,  $p \leq 0.001$ ), sodium sulfamethazine ( $t = 70.84$ ,  $p \leq 0.001$ ), sulfamethoxazol ( $t = 8.25$ ,  $p \leq 0.001$ ), and trimthoprim ( $t = 9.92$ ,  $p \leq 0.001$ ). Furthermore, the detected tylosin and sulfamethazine concentrations, in honey samples after 42 days of treatment, reached 100-120 folds of Septazol.

**Table (1):** Residues of tylosin, sulfamethazine, and septazol in the honey after 42-day of treatment.

Antibiotic applied	Residual concentration (mg/kg)	
	(Mean $\pm$ SD)	
	Control	Treated
Tylosin	0.113 $\pm$ 0.20	13.880 $\pm$ 1.415***
Sulfamethazine	0.008 $\pm$ 0.02	0.808 $\pm$ 0.013***
Septazol (Sulfamethoxazol)	0.088 $\pm$ 0.08	1.031 $\pm$ 0.182**
Septazol (Trimthoprim)	0.032 $\pm$ 0.02	1.383 $\pm$ 0.235***

\*\* , is significantly different at  $p \leq 0.01$ ; \*\*\* , significant difference at  $p \leq 0.001$ .

Other residues (Trimthoprim and sulfamethoxazol) reached to 43 and 12 fold in the honey. Generally, concentration of the residues in untreated or treated colonies is ascendingly arranged as tylosin > trimthoprim > sulfamethoxazol > sulfamethazine.



**Figure 1):** Charts of LC-MS/MS of trimthoprim (A), tylosin (B), sulfamethoxazol (C), and sulfamethazine (D) in the honey 42-day after treatment.

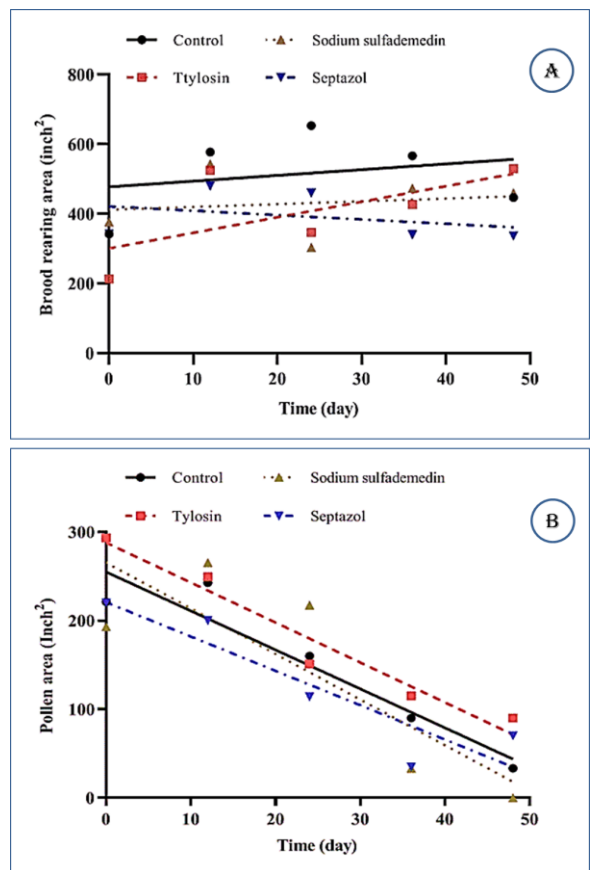
**Effect of antimicrobial drugs on the honeybee biology**

*Effect of antimicrobial drugs on brood rearing and pollen stored areas*

The effect of antimicrobial drugs on brood rearing activity is shown in Figure (2A). At 0-day, the brood-rearing area was  $342 \pm 38$ ,  $213 \pm 57$ ,  $377 \pm 22$ , and  $342 \pm 18$  inch<sup>2</sup> (M $\pm$ SD) in the control and treated colonies with tylosin, Sodium sulfademedin, and septazol, respectively. ANOVA results at 0-day indicated that treatment with tylosin recorded significantly lower ( $p \leq 0.05$ ) brood-rearing compared with all other treated groups. With time progress, the brood-rearing area indicated to follow a regression line equation:  $Y = 1.650 * X + 477.7$ ,  $Y = 4.467 * X + 301.1$ ,  $Y = 0.8056 * X + 412.0$  and  $Y = -1.256 * X + 421.9$ , in the control as well as the treated groups with tylosin, sodium sulfademedin and septazol, respectively. However, these lines are not significantly different from each other. Only tylosin was able to increase the brood-rearing area with time progress in a significant way

( $F_{1,13} = 7.74$ ,  $p \leq 0.05$ ). The brood-rearing area in the control or treated with the remaining antimicrobial drugs showed no significant changes over time.

The effect of antimicrobial drugs on the pollen area stored is shown in Figure (2B). The pollen stored area at 0-day was  $221 \pm 24$ ,  $293 \pm 32$ ,  $193 \pm 27$ , and  $220 \pm 20$  inch<sup>2</sup> (Mean  $\pm$ SD) in the control and colonies treated with tylosin, sodium sulfademedin, and Septazol. No significant differences were observed among these groups (ANOVA at 0-day). The regression analysis revealed that the pollen stored area in the control, treated colonies with tylosin, Sodium sulfademedin and septazol is inversely proportional to time increasing ( $p \leq 0.001$ ) according to the following equations:  $Y = -4.400 * X + 255.0$ ,  $Y = -4.511 * X + 288.1$ ,  $Y = -5.150 * X + 265.4$ , and  $Y = -3.875 * X + 220.8$ , respectively. However, the treatment did not affect the slopes of these lines.



**Figure 2):** Regression of brood rearing area (A) and pollen area (B) by honeybee workers against time post-treatment with several antimicrobial drugs.  $n =$  three biological replicates for each point.

*Effect of antimicrobial drugs on hygienic behavior*

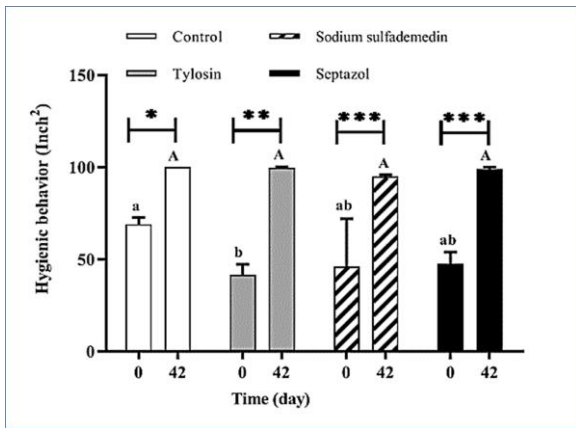
The effect of antimicrobial drugs on the percentage of removed killed brood (%) was summarized in Figure (3). Statistical Analysis (Two-way ANOVA) demonstrated that only time interval (42-day versus 0-day) affect the HB ( $F_{1,8} = 138.3$ ,  $p \leq 0.001$ ), whereas neither treatment nor the interaction affect it. The post-hoc analysis indicated that the HB at 42 days post-treatment was significantly ( $p \leq 0.05$ ) higher than those recorded at 0-day at all treatment. The Bonferroni's multiple comparisons between treatment means at 0-

day showed that the bees treated with tylosin had significantly lower ( $p \leq 0.020$ ) HB compared with the control. At 42 days post-treatment, no significant difference has been observed between the means of HB (Figure 3).

**Total reserved body reserves (protein, lipid, and carbohydrate contents)**

*Total body protein and lipid*

The effects of antimicrobial treatments on total body protein and lipids are shown in Figures 4A and B. According to the results of the pairwise comparisons, only the time recorded a highly significant reduction in total body protein and lipid contents ( $F_{1,20} = 136.7, p \leq 0.001; F_{1,20} = 231.6, p \leq 0.001$ , respectively), where, at 42 days post-treatment showed the lowest body protein and lipid contents. Neither treatment nor the interaction could affect the content of protein or lipid. Bonferroni multiple comparisons between the total protein or lipid content showed non-significant changes at 0- or 42-day post-treatment.



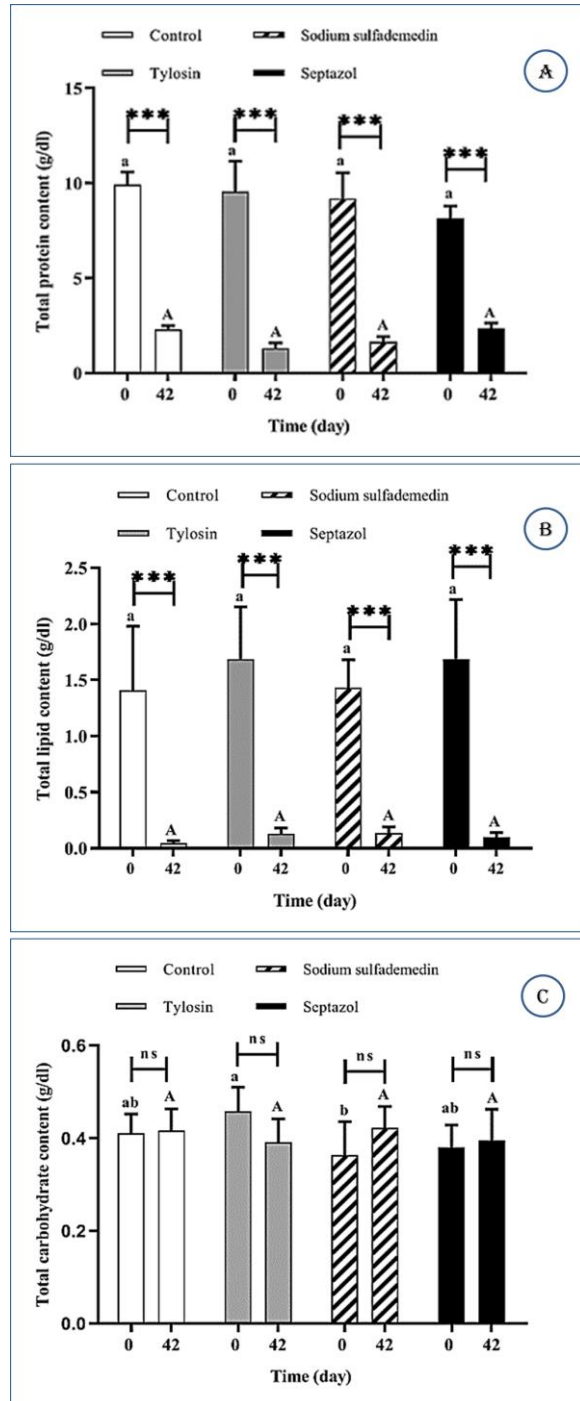
**Figure (3):** Hygienic behavior of the honeybee at 0- and 42-days post-treatment with tested antimicrobial drugs. Data are represented in mean  $\pm$ SD,  $n =$  three biological replicates per treatment. \*, \*\* & \*\*\* refer to significant differences at  $p \leq 0.05$ , 0.01 and  $\leq 0.001$  level, respectively. Bars with the same letters within each time interval are not significantly different (Bonferroni's test).

*Total body carbohydrate*

The effects of antimicrobial therapies on total body carbohydrates are shown in Figures 4C, where no significant differences over time were recorded. On the other hand, Bonferroni's multiple comparisons between the body carbohydrates among different treatments at 0-day demonstrated that the honeybee treated with sodium sulfademedin had significantly lower carbohydrates than those treated with tylosin ( $p \leq 0.05$ ).

**DISCUSSION**

Antibiotics, commonly used in apiculture, influence honey quality, honeybee biology, and physiology (Ortiz-Alvarado *et al.*, 2020). Overuse of antibiotics by beekeepers is considered to be among the reason for bees decline or CCD, which in turn affects the economy in different countries (Gallai *et al.*, 2009). Therefore, the current study addressed how antibiotic treatment commonly used affects the honey quality in terms of increased residues. The effect of these



**Figure (4):** Total body reserves (g/dl) in honeybee workers post-treatment with tested antimicrobial drugs. Protein content (A); lipid content (B); and carbohydrate content (C). Data are represented in mean  $\pm$ SD,  $n =$  three biological replicates per treatment. \*\*\* refer to significant differences at  $p \leq 0.05$ , 0.01 and  $\leq 0.001$  levels, respectively. Bars with the same letters within each time interval are not significantly different (ns) based on Bonferroni's test.

treatments on pollen area stored, brood-rearing area, HB, and metabolic reserves in the carniolian honeybee, *A. mellifera* are observed.

In this study, tylosin, trimthoprim, sulfamethoxazol, and sulfamethazin residues were found to be higher than those in the control honey at 42 days (2 generations of the worker development) after dosing. the residues concentration detected were ascendingly arranged as tylosin > trimthoprim > sulfamethoxazol > sulfamethazine. The results of tylosin are slightly

higher than those recorded by Adams *et al.* (2007) who found tylosin residues equal to 17 mg/kg 3 days after dosing. In the control samples, the means recorded were 0.113, 0.088, and 0.008 mg/kg for tylosin, sulfamethoxazol, and sulfademedin respectively. These concentrations were consistent with those reported by Solomon *et al.* (2006) who analyzed 3855 samples and showed that tylosin residues were in the range 2-18 µg/kg and sulphonamid in the range 4.6-5 µg/kg.

In this study, all samples recorded higher values of the residues 42-day post-application. As a result, since the influence of antimicrobials tylosin, sodium sulfademedin, and septazol (understudy) on honey biology and metabolic reserves was observed, we recommend that beekeepers design a new approach for managing honeybee infections in Egypt.

The results obtained herein demonstrated that only tylosin was able to reduce the brood-rearing area and HB at 0-day. Tylosin has an acute effect on honeybee activity. This may reflect the stressful conditions or reduced vigor at this treatment as reported by Güler and Toy (2013). Bacandritsos *et al.* (2007) observed that HB decreased on exposure to oxalic acid. Ismail *et al.* (2006) recorded that treatments with eugenol reduced brood rearing activity. Moreover, Daisley *et al.* (2020) recorded a decrease in brood rearing after oxytetracycline application and showed a significant increase in brood count on adding LX3 select strains of 3 lactobacilli (*Lactobacillus rhamnosus* GR-1, *Lactobacillus plantarum* Lp39, and *Lactobacillus kunkeei* BR-1; LX3) to treated bees. Ortiz-Alvarado *et al.* (2020) observed that exposed bees to antibiotic treatment, induced a prolonged adiposity peak, stayed nurses, and delayed behavioral development into foragers (pollen collector). The results indicated that the HB at 42 days post-treatment was similar to the control value recorded. Abou-Shaara *et al.* (2013) reported that foraging activity is impacted by colony strength and brood-rearing activity. These investigations explain why pollen area stored decreased with time in treated groups. Keller *et al.* (2005) and Brodschneider and Crailsheim (2010) reported that pollen is not stored in large quantities and is used depending on floral resource availability and the colony's needs. At the end of the experiment, we noticed that tylosin induced an increase of brood rearing area with time elapsed. This may refer to the half-life time of tylosin residue within *A. mellifera*. The half-lives of tylosin in honey at 34 °C equal 130 days. Tylosin degraded to desmycosin in honey in mildly acidic conditions pH < 4 (Kochansky, 2004). Otherwise, the dosage of sodium sulfademedin and septazol could not affect the brood-rearing area in the treated honeybees.

Our results showed non-significant differences in total body protein, lipid, and carbohydrate among treatments. However, the total body protein and lipids were lower at 42 days than those at 0-time at all conditions tested. In agreement, Crailsheim (1990) recorded that level of protein varied with season and availability of food outside the hive. Feazel-Orr *et*

*al.* (2016) observed no effect on body weight, protein, and carbohydrate levels in treated bees with tau-fluvalinate. (Loucif-Ayad *et al.*, 2010) observed reduced amounts of hemolymph protein, carbohydrate, and lipid in colonies treated with Bayvarol and Apivar, while no significant difference among colonies treated with Apiguard, ApiLifeVar and untreated colonies. Our results showed that treatment with sodium sulfademedin reduced the carbohydrates contents at 0-day compared with the control. Similarly, Adjlane *et al.* (2013) reported that treatment with oxalic acid disrupted the metabolism of the bee.

## CONCLUSION

According to this study, the common antibacterial dose employed by Egyptian beekeepers could result in a significant percentage of residues in honey. Except for tylosin, the same dose had no effect on the honeybee's brood area, pollen area, or HB. On total body proteins, lipids, and carbs, no additional impacts were seen. Future research should focus on the survival and immune competence of treated honeybees, particularly when a link to the gut microbiome is discovered.

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## تأثير بعض المضادات الميكروبية على الكفاءة الحياتية لنحل العسل *Apis mellifera* I (Hymenoptera: Apidae)

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### الملخص العربي

يستخدم النحالين عدد من المضادات الميكروبية في مكافحة الأمراض البكتيرية و الفطرية في نحل العسل. هذا يؤثر مخاوف حول العالم لما قد يكون له تأثير علي صحة الانسان. لذلك تهدف هذه الدراسة إلى تقييم التركيزات المنبقيه لثلاث من المضادات الميكروبية شائعة الاستخدام وهي التيلوزين و سلفاديميدين الصديوم و السبيبتازول التي تستخدم لعلاج أمراض نحل العسل، وكذلك دراسة تأثير هذه العقاقير على بيولوجيا النحل. تم تجميع عينات من عسل النحل بعد (مدة جيلين من تطور الشغالة) 42 يوم من إنتهاء المعالجة للتحليل باستخدام HPLC، كما تم ملاحظة نشاطات النحل (مساحة تربية الحضنة و حبوب اللقاح) عند 5 فترات زمنية كل 12 يوم من إنتهاء المعالجة. أما سلوك التنظيف و المخزونات الأيضية (بروتينات و دهون و كربوهيدرات الجسم) فقد تم ملاحظتها عند فترتين فقط (0 و 42 يوم بعد المعالجة). أظهرت النتائج أن بقايا العقاقير المضادة للميكروبات كانت أعلى في العسل الناتج من الطوائف المعاملة عنها من المجموعة الضابطة (الطوائف الغير معالجة). كما أوضحت النتائج عدم وجود تأثيرات ضارة على نشاطات مساحات تربية الحضنة و حبوب اللقاح و سلوك التنظيف لنحل العسل المعامل بالجرعات المقررة. في نفس الأثناء أظهرت المجموعة المعاملة بالتيلوزين إنخفاض في سلوك التنظيف عن المجموعة الضابطة عند الزمن (صفر) أي بعد 12 يوم من بدء العاملة. وكذلك فإن مرور الزمن لم يؤثر على مساحة تربية الحضنة بعد كل المعاملات. ومن الناحية الفسيولوجية ، لم تظهر المخزونات الأيضية للجسم أي إختلاف معنوي بعد المعاملة. مع ذلك كانت البروتينات و الدهون أقل بعد 42 يوم من إنتهاء المعاملة مع تلك التي سجلت عند الزمن (صفر). وبناء على هذه النتائج ، فقد خلصت الدراسة إلى أن العسل الناتج من النحل المعامل بالمضادات الميكروبية محل الدراسة إحتوى على خطر التلوث الناتج عن الممارسات الخاطئة للنحالين وكذا خطر محتمل على نحل العسل ذاته.