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Original article

Antibiotic treatment (Tetracycline) effect on bio-efficiency of the larvae honey bee (*Apis mellifera jemenatica*)

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ABSTRACT

Honey bees are important for ecological health, biodiversity preservation, and crop output. Antimicrobials, like Tetracyclines, are commonly used in agriculture, medicine, and beekeeping, bees might be exposed to Tetracycline residues in the environment either directly or indirectly. This study aimed to determine the effect of antibiotic treatment (Tetracycline) effect on the Bio-efficiency of the larvae honey bee (*Apis mellifera jemenatica*), when larvae honeybee workers were exposed to different concentrations of it, to see how long they survived after being exposed to it and affected this antibiotic to the histological structure of the midgut. The results demonstrated that the concentration (LC_{50} = 125.25 µg/ml) of antibiotics Tetracycline leads to kills half of the individuals. Our data indicate that the high concentrations of Tetracycline have a significant effect on the histological composition of the cells of the midgut of honeybee larvae. Antibiotic exposure can negatively impact the health of honey bees, especially Tetracycline because it is the most used antibiotic in apiculture.

1. Introduction

Honey bees are crucial for global ecological stability and agricultural output, being the most economically significant pollinator. But the global loss of colonies in the last decade has put agricultural productivity and food security at risk (Garibaldi et al., 2011). The general decrease in colony numbers of native bees and wild bees, and the increase in colony losses every year are now quite concerned (Serra et al., 2021). Invasive pests and/or pathogenic products (e.g. varroa, nosema), agricultural pesticides (e.g. neonicotinoids), and scarcity of native bees and honeybees are the drivers connected but not limited to such declines (Cameron et al., 2011; Smith et al., 2013; Wilfert et al., 2016). These factors may influence bee health individually or in combination (Smith et al., 2013).

Since 2006, the mortality of honeybees throughout the globe has increased, as a consequence of several factors (Potts et al., 2010). This insect is threatened with several illnesses, including bacteria, fungi, viruses, and protozoa. The Positive Gram bacteria *Penibacillus larvae* and *Melissocccus plutonius* are responsible for both the American foulbrood (AFB) and the European foulbrood (EFB) (Masry et al., 2014). As a result, P. larvae are causing substantial economic losses to honeybee colonies. Because of the disease's destructive consequences, beekeepers are required to burn their hives and disinfect their equipment in the event of an epidemic of the (AFB) (Mutinelli, 2003).

Researchers have been testing a variety of antibiotics to combat (AFB) (Katznelson, 1950). Aureomycin (Chlortetracycline), followed by penicillin, chloramphenicol, streptomycin, and other antibiotics, was the most efficient antibiotic against P. larves, the ongoing use of antibiotics in P. *larvae* has created antibiotic resistance. In addition, heavy Oxytetracycline (OTC) usage creates a deposit in bee products including honey, pollen, and beeswax (Kochansky et al., 2001). Oxytetracycline (OTC) was recently banned in a number of nations for these reasons (Mutinelli, 2003). For the past years, antibiotics from the Tetracycline class, such as Tetracycline and the related chemical Oxytetracycline, have been the chosen treatment for AFB in the United States. Although P. larvae exclusively infect larvae, the antibiotic is also consumed by non-target adult bees during preventive therapy (Bulson et al., 2021).

Furthermore, the presence of antibiotic resistance genes (ARGs) inside the honey bee colony, including within P. larvae, has been

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linked to prophylactic Tetracycline treatment of bees (Tian et al., 2012). In addition to reducing the efficacy of Tetracycline in honey-bee treatment of American Foulbrood disease, this also increases the worldwide reservoirs that ARGs available for human infections can acquire (Allen et al., 2010; Hu et al., 2017). The excessive use of Tetracyclines, however, though necessary in some situations, that pollutes the environment and involves an uneventful buildup of antibiotic resistance genes that are important to human and honey bee's health (Tian et al., 2012). This jeopardizes the overall health of hives, as the unique microbiota found in healthy bees is critical for metabolic competency, immunological modulation, growth and development, and parasite and pathogen resistance (Raymann et al., 2018).

Many studies linked the honey bee's health to efficiently the digestive system, which is an important organ for honey bee health since it is where pathogens and xenobiotics come into contact on it (Raymann et al., 2017). The detoxification of ingested xenobiotics is carried out by the midgut Epithelium (Higes et al., 2013). Because the midgut is responsible for the digestion and absorption of ingested food, it is an important organ for toxicity analysis. The midgut is the only tissue of adult honey bees that exhibits widespread cell proliferation (Ward et al., 2008). According to numerous scientists, the food channel (midgut) is the main tissue through which metals are collected (Zhang et al., 2001). The effects of light and electron microscopes on the histological and cellular structure of midgut cells were examined and percentage of apoptosis and/or necrose incidence were also measured by flow cytometry in midgut cells (Dabour et al., 2019). Furthermore, it is one of the most common points where injected metals come into touch with bees (Catae et al., 2014).

And another study conducted by (Raymann et al., 2017) found that exposing worker bees to a therapeutic dose of Tetracycline significantly changed their gut microbiome abundance and community composition, as well as increasing their mortality. They determined that the fitness drop was not attributable to Tetracycline's direct toxicity on the infested workers, but by treating germ-free workers with the antibiotic. Therefore, maintaining the health of honey bees from all environmental pollutants that may harm them is one of the most important aspects to focus on, especially since many studies consider antibiotic Tetracycline-based agents to be of particular concern due to their widespread use in human medicine and as bacteriostatic feed additives in livestock. So, this study aimed to investigate of antibiotic (Tetracycline) the effects at different concentrations (0.015, 0.15, 1.5, 15, and 150 μg/ml) on the larva honey bees (*Apis mellifera jemenatica*), effect on the Bio-efficiency, in terms of knowing how long they survived after being exposed to it, also on the histological structure of the midgut by light microscope and scanning electron microscope.

2. Material and methods

2.1. Individuals used in the study

In May 2021, experimental honeybees from healthy colonies in the apiary were collected of King Abdulaziz University's Faculty of Environmental Sciences research station. The following procedure was used to obtain the test larvae. Three typical Queen laying eggs were inserted in empty combs and deposited eggs for eight hours in a queen egg laying controller; combs holding newly put eggs were then moved by a Queen excluder separately to the same site. The comb was promptly moved to the entomology laboratory, and four days later, it was kept in an incubator at 34 ± 1 °C.

Material and food administration

Jensen et al., (Jensen et al., 2009) methods' of rearing the larvae was used with a few modifications. The two-day larvae were randomly transferred onto six 48-well plates with a false 10 L feed in each well using this grafting tool. The control group consisted of three plates, while the treatment group consisted of three plates on the left. In Vojvodic et al., the artificial diet formula was referred to (Vojvodic et al., 2011). As described by Nie et al., (Nie et al., 2020) these larvae were fed once a day based on the quantity. The plates were incubated at 34 ± 1 ℃ in a dark incubator with relative humanity of 95 _ 2%. In the treatment group, larvae were fed a meal containing various antibiotic concentrations (Tetracycline) were orally exposed at concentrations (0.015, 0.15, 1.5, 15, and 150 μg/ml). Then mortality was assessed and determined the Lethal concentration (LC_{50}). The larvae in the control group were fed a normal diet without Tetracycline (Duan et al., 2021).

2.2. Antibiotic treatment of A. Mellifera larvae

The antibiotic (Tetracycline):

Tetracyclines (TCs) are the most widely used antibiotics on the planet. This broad-spectrum antibiotic family has been known for the suppression of protein synthesis in bacteria in addition to combating several bacterial illnesses. The basic structural element is a tetracyclic ring with various functional groups of hydroxyl, methyl, keto, and dimethylamino, (Fiaz et al., 2021), Fig. 1.

2.3. Methods of study

Honeybee workers in this research (*Apis mellifera jemenatica*) were exposed to different concentrations of the antibiotic (Tetracycline) to see how long they survived after being exposed to Tetracycline, as well as the midgut's histological structure.

2.3.1. The mortality

Honey bee workers larvae were exposed to four different concentrations of the antibiotic (Tetracycline) orally exposed, the number of individuals used in the study was 60 larvae and they were followed up within 48 h, the mortality was assessed at (0.015, 0.15, 1.5, 15, and 150 μg/ml) (Gokulan et al. 2017) and determined the Lethal concentration (LC_{50}). On the other hand, control group (0.00 μg/ml), were fed normally and not exposed to antibiotic(Tetracycline).

2.3.2. The histological study of the midgut

A group of honey bee workers larvae was exposed to concentration (LC_{50} = 125.25 μg/ml) for five days, and then examined the histological composition of the stomach. In addition to a control group (0.00 μg/ml).

2.3.2.1. Light Microscope (LM). The samples for the histological examinations were produced using the historesin procedure as follows: Five-day-old larvae were collected and preserved in a preservation solution. Were fixed in a 0.1 M phosphate buffer solution containing 10% paraformaldehyde (PH 7.2). The material was dehydrated in an escalating sequence of 20-minute ethanol baths at 70,

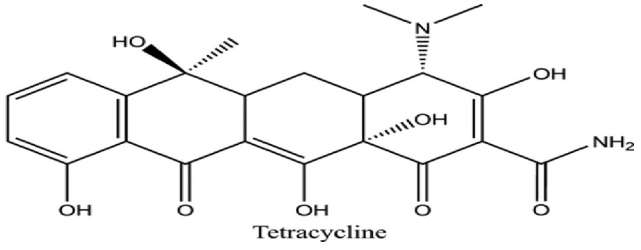

Fig. 1. Chemical structure of Tetracycline.

80, 90, and 95 percent ethanol, then transferred to the resin solution for 72 h at 4oC. In the end, the mixture was put into molds that were then filled with a catalytic resin and closed with a microtome bracket. The blocks were sliced with a microtome after polymerization. The slices were mounted on slides with hematoxyline and eosin stained. Samples were then investigated and photographed using a microscope for Olympus (Olympus-Bx41) (Aljedani, 2018; Serra et al., 2021).

2.3.2.2. Scanning electron microscope (SEM). Larvae samples have been fixed in the solution of glutaraldehyde containing 2.5 percent glutaraldehyde for 1/2 to 1 hr. After that, the specimens rinse larvae midgut three times in distilled water for 10 min. Then fixed in Osmium Tetroxide for 1 hr. Rinse tissue twice in distilled water for 1 min. In a graded ethanol series, 50% Ethanol for 1 min, then 100% Ethanol for 10 min, and Ethanol: Acetone (1:1) for 10 min. After that pure acetone three changes for 10 min. Then it is transferred to Acetone: Hexamethyldisilazane (1:1) for 1 hr. and put in pure Hexamethyldisilazane for 1 hr. Remove the Hexamethyldisilazane and put the sample under vacuum for 10 min (H.P). The specimens were gold-sputtered for 60 s using an Auto Fine Coater (JFC-1600) and viewed at 10 kV with a Quanta-250 Scanning Electron Microscope in King Fahd Medical Research Center in King Abdulaziz University.

2.4. Statistical analysis

After determining the corrected rate of mortality using Abbott's formula (Abbott, 1925). The LC_{50} values were calculated using mortality regression lines drawn by the Finney, (1971) method and by a program of Bakr, (2010)Ldp line. SPSS 22.0 software (IBM, Armonk, NY, USA) was used for all statistical analyses. The mean standard error is used to present all data (S.E.) (SPSS., 2013).

3. Results

3.1. The mortality

Honey bee workers larvare were orally exposed to four different concentrations of the antibiotic (Tetracycline). The mortality was assessed and determined the Lethal concentration (LC_{50}) at (0.015, 0.15, 1.5, 15, and 150 g/ml for 48 h). The number of dead individuals was 2 larvae within 48 h, which is within the normal limits.

In this study, laboratory toxicity was evaluated for Tetracycline, of this antibiotic 48 h after the treatment of the larvae honeybee worker by Tetracycline. The toxicological effects of Tetracycline were examined, and it was discovered that there was a direct correlation between the tested concentrations and the death percentages of the treated larvae honeybee workers. The results also showed that the effective concentrations of the Tetracycline were (0.015, 0.15, 1.5, 15, and 150 μg/ml). Death rates for treated larvae honeybee ranged from 7.143 % for the lowest concentration to 54.082 % for the highest concentration in Table 1.

On the other hand, the laboratory toxicological lines of the Tetracycline and the statistical constants derived from them showed the difference of the lethal concentration's values for half of the number of tested larvae honeybee 25%, 50 %and for 90%, which is known as LC_{25}, LC_{50}, and LC_{90}, and treatment with the Tetracycline (2.472, 125.2544 and 217234.8 μg/ml) respectively. The confidence intervals ranged from the minimum to the upper limit corresponding to these concentrations from (1.1138, 5.1841 μg/ml), (48.0234, 506.2756 μg/ml), and (23097.68, 8105668 μg/ml) respectively, at a 95% confidence, Table 2.

Evaluation of the values of concentrations of antibiotic that cause death of 50% for larvae honeybee which is what is known as LC_{50} reached to (LC_{50} = 125.25 μg/ml) Figs. 2. and 3.

3.2. The histological study of the midgut

A group of the larvae honey bee workers was exposed to the antibiotic (Tetracycline) at a concentration (LC_{50} = 125.25 μg/ml) for five days, and then histological composition of the midgut was studied. In addition to a control group (0.00 μg/ml).

3.2.1. Light Microscope (LM)

Results of the current study showed that after five days, providing normal food to the midgut(mid) of the 5th larval instar honey bee worker (the control), possess Epithelium (Epth) and a normal Nucleus(Nu), the entire and regular cell boundary with homogeneous cytoplasm inclusion has a striated border (Sb) at the apex. Column cells organized into one layer are the most prevalent epithelial cell type and settled on the basement membrane (Bm), another type of Epithelium that could be observed is regenerative cells (Rg) their terminals did not reach the lumen(L). Also, the spread of cytoplasmic vacuoles (Vacu) was observed in Epithelium columnar, as well as, the presence of Epithelium layer cells of the proliferating small Epithelium digestive cells (Pf) as small buds arises from the stomach Epithelium, which arises from the division of Epithelium cells. Midgut Epithelium bordered by an external muscle layer (Cmscl) and an outer longitudinal (Lmscl), as described in (Fig. 4 (A)(B)(C)(D)); In the present study, the columnar Epithelium cells appeared (Epth) in multiple and cumulative layers on top of each other, and the cell membrane is affected after exposure to the antibiotic (Tetracycline) at a concentration (LC_{50} = 125.25 μg/ml) for five days, also the cells not more clearly with the lack of boundaries between cells and rupturing the cell membrane and cell components mixture with each other, also the border of the cell was not well, and the cell membrane affected, the spread of cytoplasmic vacuoles (Vacu) was observed in Epithelium columnar exposed cells to Tetracycline and observed their presence heavily at bases of cells, and on the tops of cells, The regenerative cells (Rg) present and developing clearly beside cells bases in the control group, while in the Tetracycline it is not clear cell border and presence at bases of Epithelium cells are not negligible and demonstrated a varying degree of growth.

The presence of an Epithelium layer cells of the proliferating small Epithelium digestive cells (Pf) as small buds arises from Epithelium stomach, which arises from the division of Epithelium

Table 1
Relation between different concentrations of Tetracycline and mortality of the larvae honeybee worker's death after 48 h of treatment.

Linear probit	Linear response%	Observed response %	Log (Con. * 10)	Con. (μg/ml)
0.000	0.000	2.000	0.000	0.00
3.4483	6.03962	7.143	0.1761	0.015
3.844	12.3907	10.204	1.1761	0.15
4.2397	22.355	25.51	2.1761	1.5
4.6353	35.7694	30.612	3.1761	15
5.031	51.2342	54.082	4.1761	150

Table 2
LC_{25}, LC_{50}, and LC_{90} values, slope values for Tetracycline toxicity Line, and Maximum and Minimum values.

Slope	Chi	r	LC_{25}	LC_{50}	LC_{90}
0.3957	2.7185	0.9821	2.472	125.2544	217234.8
+/- 0.0492	tabulated 7.8	tabulated 0.878			
Lower limit (μg/ml)			1.1138	48.0234	23097.68
Upper limit (μg/ml)			5.1841	506.2756	8,105,668

Chi-Square (Chiinv) (Chi), probability (p), correlation coefficients (r).

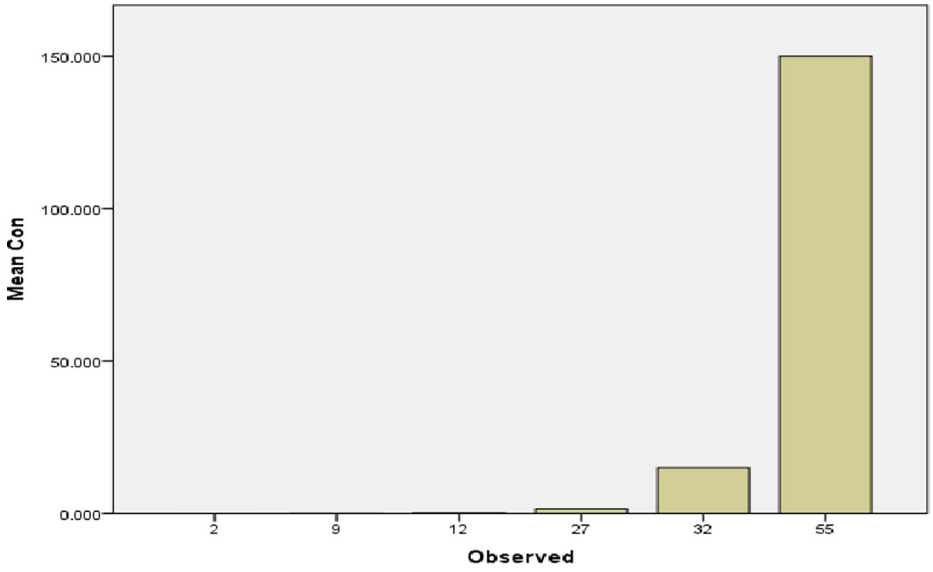

Fig. 2. Showed relation between different concentrations of Tetracycline and observed response values on a larvae honeybee worker after 48 h of exposure.

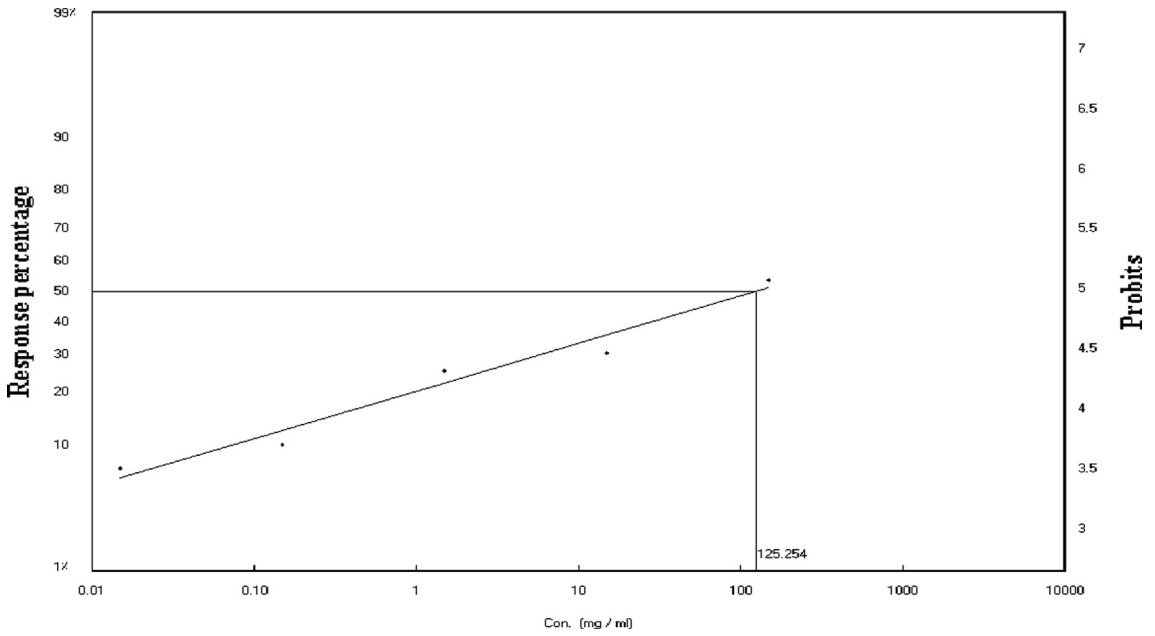

Fig. 3. Showed Lethal concentration (LC_{50}) values when honeybee workers larvae after 48 h of exposure to different concentrations of Tetracycline.

cells present in the case of the control, while in exposure to Tetracycline there are large numbers of it at the tops of the cells.

When exposed midgut Epithelium cells to Tetracycline the peritrophic membrane(Pm) was analyzes and decomposition while normal in control group. Epithelium cells stabilize on the basement membrane(Bm), and the cell membranes are completely decayed especially in the case of Tetracycline, and the outside layer of muscles that seem profligate when exposed in the case of all Tetracycline. The midgut epithelial tissue of the bees orally treated with the LC_{50} concentration of Tetracycline appeared to be affected under the action of this antibiotic. Their nuclei are abnormal, bounded by large vacuoles, the cytoplasm is characterized by

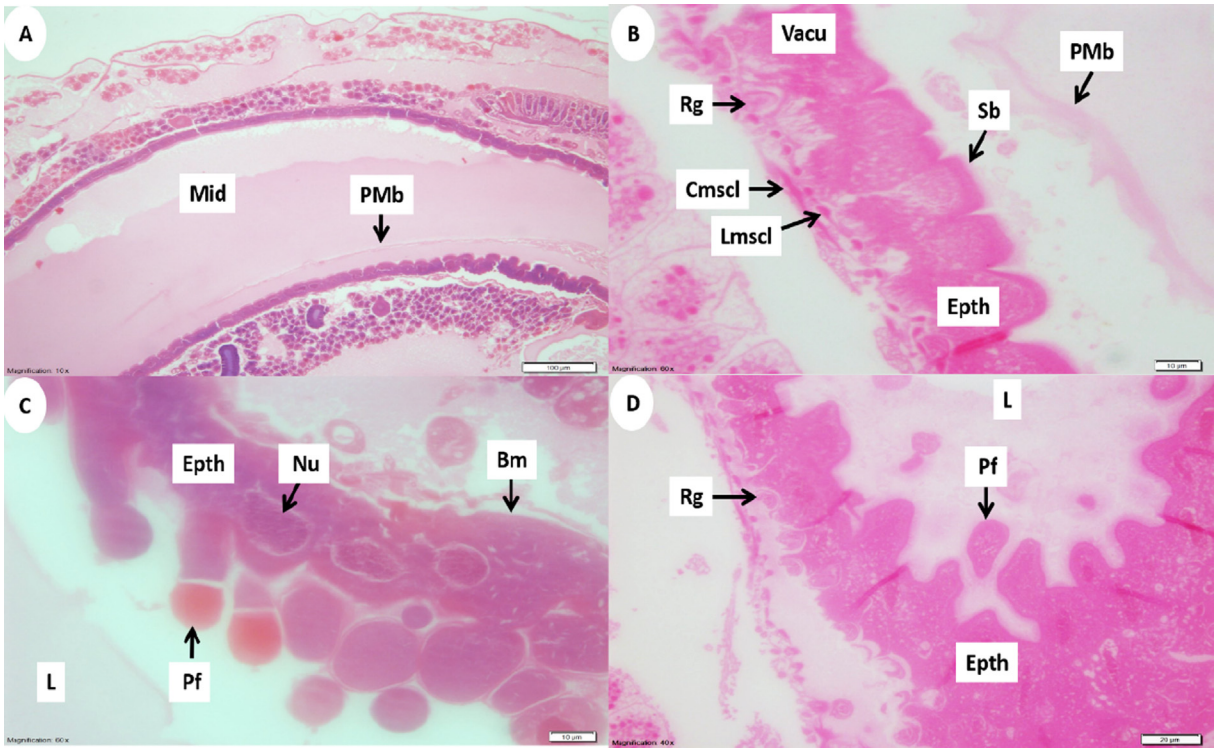

Fig. 4. Longitudinal section of the midgut(Mid) in the 5th larval instar honey bee worker after 5 days of control group by Light Microscope (LM): A (X - 100) B (X – 10) C(X-10)D(X-20);Bm: basement membrane, Cmscl: circular muscles, Epth: Epithelium cells, L:lumen, Lmscl: longitudinal mucles, Nu: nucleus, PMb: peritrophic membrane, Pf: proliferating small Epithelium digestive cells, Rg: regenerative cells, Sb: striated border., Vacu: vacuoles.

dense, coarse, and scattered inclusion, the cell membrane is not affected and appears to be still intact for the control group. The nuclei have disappeared or diminished, the cytoplasm inclusion is scattered, and the cell membranes are decayed completely as shown in the group treated with Tetracycline. Some cells were separated from the basement membrane. The present study showed that Tetracycline was more harmful to midgut Epithelium tissue in honey bee larvae, impacted the histological structure of the midgut. (Fig. 5 (A) (B) (C) (D)).

3.2.2. Scanning Electron Microscope (SEM)

A group that is not exposed (control)

The results of this study showed that the midgut (stomach) is a member in the abdominal cavity that following the foregut in the larval honey bee worker. The cavity is divided into many sections of the midgut wall (lumen (L)). The midgut is made up of the articulated larval honey bees, which feed on regular food (not antibiotic (control)), and the Epithelial cells (Epth) consist mostly of columnar digestive cells which look big in cells during proliferation, with a tiny gap or cytoplasmic holes (vacuoles). The epithelial tissue normally develops through morphological changes as digestive cells die, in the honeybee midgut. The cavities are extended into the inner cavity, and the cilia epithelial striated border (Sb) with many holes and curves are found at the top. The cells were founded on the basement membrane (Bm), the anchor of all cells. On the outside surround the midgut with two layers of muscle: inner circular muscles (Cmscl) and outer longitudinal muscles (Lmscl), Fig. 6 (A,B,C,D,E,F).

An exposed to antibiotic group (Tetracycline):

Results of the current study on the antibiotic impact (Tetracycline) at a concentration (LC_{50} = 125.25 μg/ml) showed that Tetracycline made midgut lumen (lumen (L) less spatial in the five days larval honey bee worker, Tetracycline had a greater impact on the luminal average on the inside scoop. The epithelial cells became continuous with one another, and the holes or gaps caused by curves on their striated border vanished nearly completely. The epithelial layer is affected by Tetracycline exposure, as the cells fuse.

When digestive cells die, morphological changes developed in the midgut epithelial tissue. The cavities are extended into the inner cavity, and the cilia epithelial striated border (Sb) with many holes and curves are found at the top. The basement membrane (Bm) of the cells was the basis and anchor for all cells, as well as there are two layers of muscles the outer muscles (circular) (Cmscl) and the interior muscles longitudinal (Lmscl) that surround the midgut. Tetracycline appears to have induced various alterations, including detachment from the basal membrane, in the natural condition. And the muscular layers appear to have been crushed as a result (Fig. 7(A,B,C,D,E,F).

4. Discussion

The scientific community, beekeepers, and the general public were concerned about the sudden drop in bee populations. Raising awareness of the problem has resulted in the creation of numerous techniques aimed at conserving beehives and restoring or increasing the population of honey bees. However, reducing the impact of pollution on honey bees is vital not just for the insects' well-being and the value of the honey they produce for beekeepers, but also for the value of pollination, which many key crops rely on. Previous studies have shown that climate change and/or human activity can influence food availability and diversity (Ziska et al., 2016). Pathogens have also been identified as one of the most significant causes of the global loss of honeybees (*Apis mellifera*) and other forest pollinators that sustainable Agriculture and the global food supply (Goulson et al., 2015). Honey bees are an important reservoir of enzootic diseases that can alter disease epidemiology in diverse

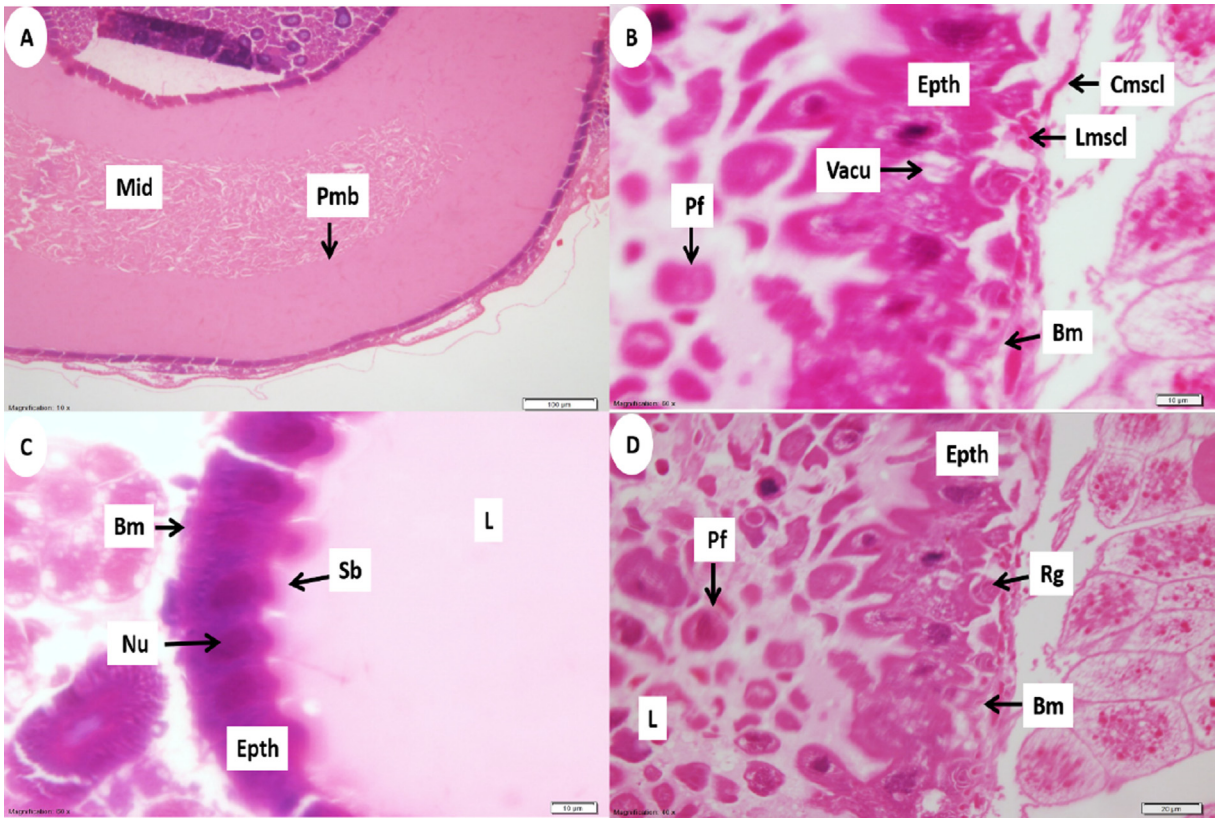

Fig. 5. Longitudinal section of the midgut(Mid) in the 5th larval instar honey bee worker after 5 days of Tetracycline group by Light Microscope (LM): A (X-100) B (X-10)C(X −10)D(X −20); Bm: basement membrane, Cmscl: circular muscles, Epth: Epithelium cells, L:lumen, Lmscl: longitudinal mucles, Nu: nucleus, PMb: peritrophic membrane, Pf: proliferating small Epithelium digestive cells, Rg: regenerative cells, Sb: striated border., Vacu: vacuoles.

animal species, and managed honey bees make up a significant portion of total pollinators(Fürst et al., 2014). To address this problem, beekeepers regularly treat their hives with antibiotics in an attempt to prevent or minimize disease and manage intraspecies pathogen transmission across apiaries (Daisley et al., 2020).

In the present study honeybee workers larvae were exposed to four different concentrations of the antibiotic (Tetracycline) orally. The mortality was assessed and determined the Lethal concentration (LC_{50}) at (0.015, 0.15, 1.5, 15, and 150 µg/ml for 48 h), While control group (0.00 µg/ml) larvae were fed the normal diet without Tetracycline. From the results of the current study, it is clear that the laboratory toxicological lines of the Tetracycline and the statistical constants derived from them showed a difference of the lethal concentration's values for half of the number of tested honeybee larvae at 25%, 50 %and for 90%, which is known as LC_{25}, LC_{50} and LC_{90}, and treatment with the Tetracycline (2.472, 125.2544 and 217234.8 µg/ml) respectively. The confidence intervals ranged from the minimum to the upper limit corresponding to these concentrations from (1.1138, 5.1841 µg/ml),(48.0234, 506.2756 µg/ml), and (23097.68, 8105668 µg/ml) respectively, at a 95% confidence. On the other hand, in a control group, the number of dead individuals was 2 larvae within 48 h, which is within the normal limits.

This is in line with the research results (Raymann et al., 2017) antibiotic exposure has resulted in reduced survival, both in hives and in laboratory experiments exposing bees to opportunistic bacterial infections, and its results showed that antibiotic exposure dysbiosis impacts bee health. Generally, antibiotic treatment of bee colonies has been routinely used in the United States for over 50 years to prevent foulbrood, a bacterial illness of bee larvae (Genersch, 2010). The two antibiotics that beekeepers most frequently use are Tetracycline (or the related chemical Oxytetracycline) and Tylosin. Tetracyclines are also used in individuals to treat bacterial conditions and often used in livestock feed which lead to resistance to Tetracycline in various bacteria, including dangerous ones (Armoni et al., 2002). However, for apiculture as part of best practice, prophylactic use of Oxytetracycline (OTC) is proposed in several parts of the world (Daisley et al., 2020).

This agrees with (Morfin et al., 2020) where they found that clothianidin decreased the weight of the bee at the highest dose and weight considerably and there was a cross-interaction between 2 wt stressors that meant abiotic stressors can have complex interactions, including the additive efficacy of the bees, when three sublethal doses of clothianidin were administered to the honeybee. Furthermore, this agrees with (Dai et al., 2018) To investigate the effects of glyphosate on *Apis mellifera* mortality, developmental rate, larval weight, and midgut bacterial diversity in the lab, larvae were grown in vitro and fed a diet containing glyphosate at concentrations of 0.8, 4, and 20 mg/L. Negative and positive controls (dimethoate 45 mg/L) were used to compare the dependent variables. Brood survival was lower in the 4 and 20 mg/L glyphosate treatments but not in the 0.8 mg/L treatments, and larval weight was lower in the 0.8 and 4 mg/L glyphosate treatments. Although the developmental rate was unaffected by exposure to three dosages, the researchers concluded that high glyphosate concentrations are harmful to young bees.

The composition and quantity of gut microorganisms are expected to influence many members of the gut community, such as the Tetracycline (Tian et al., 2012). Likewise, the use of Tetracycline in U.S. beekeeping has contributed to the buildup of resistance genes in US microbiomas of honeybees in nations that do not use antibiotics in apiculture (Tian et al., 2012). Our data indi-

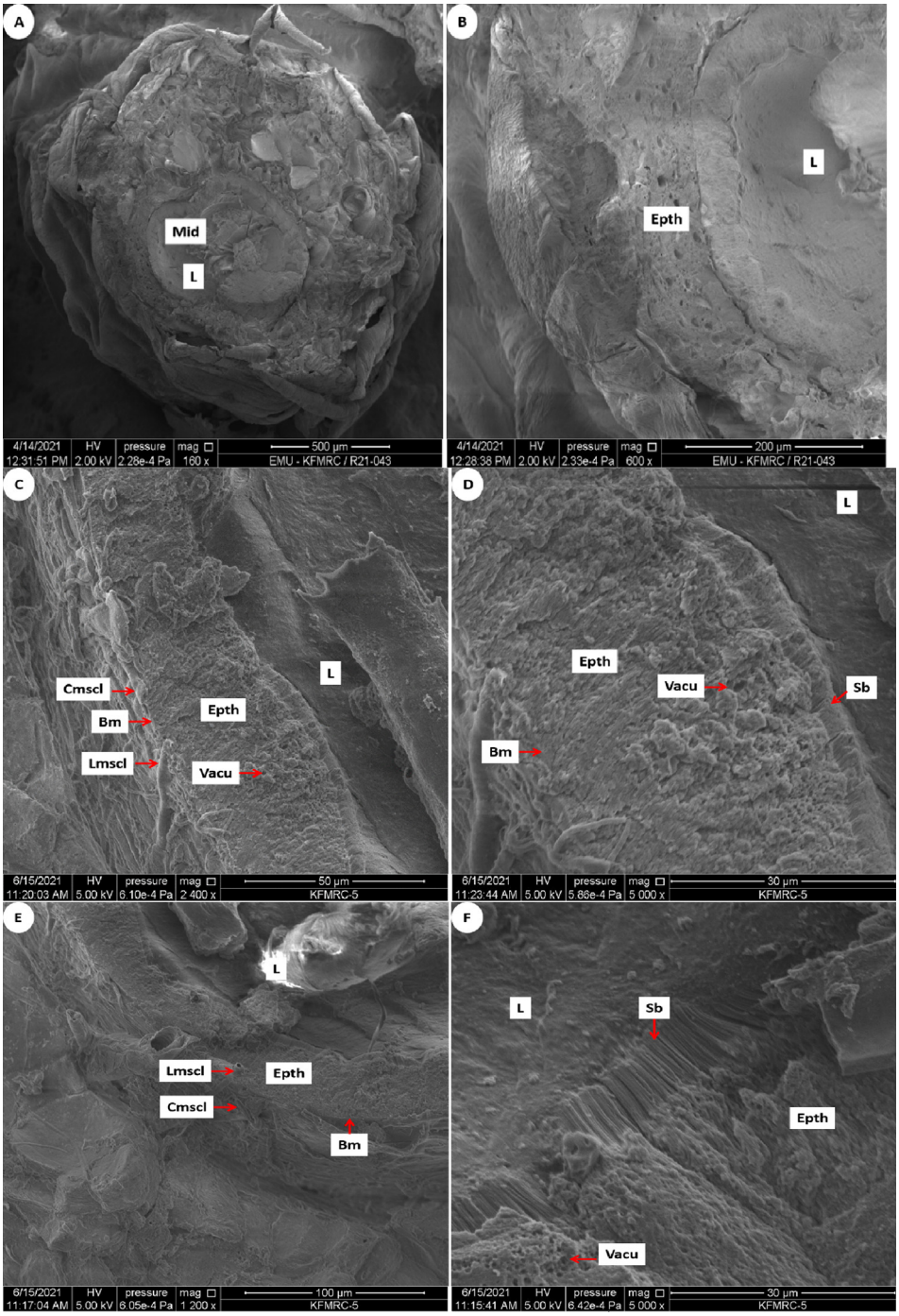

Fig. 6. cross section of the midgut(Mid) in the 5th larval instar honey bee worker after 5 days of control group by Scanning Electron Microscope (SEM): A (X −100) B (X-10) C (X- 10)D (X − 20) E(X- 100)F (X- 400); Bm: basement membrane, Cmscl: circular muscles, Epth: Epithelium cells, L:lumen, Lmscl: longitudinal mucles, Sb: striated border., Vacu: vacuoles.

cate that the concentration (LC_{50} = 125.25 µg/ml) of Tetracycline have a significant effect on the histological composition of the cells of the midgut of larvae honey bee workers, but, a control group (0.00 mg/L) did not show any change in the histological composition of the cells of the midgut of it.

Antibiotic usage has been linked to changes in the microbiomes of humans and livestock in several studies (Robinson et al., 2010). Gut communities have been linked to both nutrition and disease defense in honeybees and bumblebees, two globally important pollinators (Schwarz et al., 2016). This is evident from the results of a study by (Gregorc and Ellis, 2011) the midgut Epithelium of adult honey bees is the only tissue that demonstrates extensive cell growth, and when the midgut Epithelium of honey bee larvae was exposed to sublethal amounts of a wide range of pesticides, increased apoptosis was observed. Proliferation is also found in the midguts of stingless adult bees, however at a slower rate than that seen in honey bees (Fernandes et al., 2010). Willard et al., 2011, found that Toxic consequences can enhance the growth rate, on the one hand, by raising the cellular substitution need. When the replicative capacity of the intestinal stem cells (ISCs) isn't sufficient, the epithelial function could be harmed and the longevity decreased, on other hand, toxicants could cause direct damage to

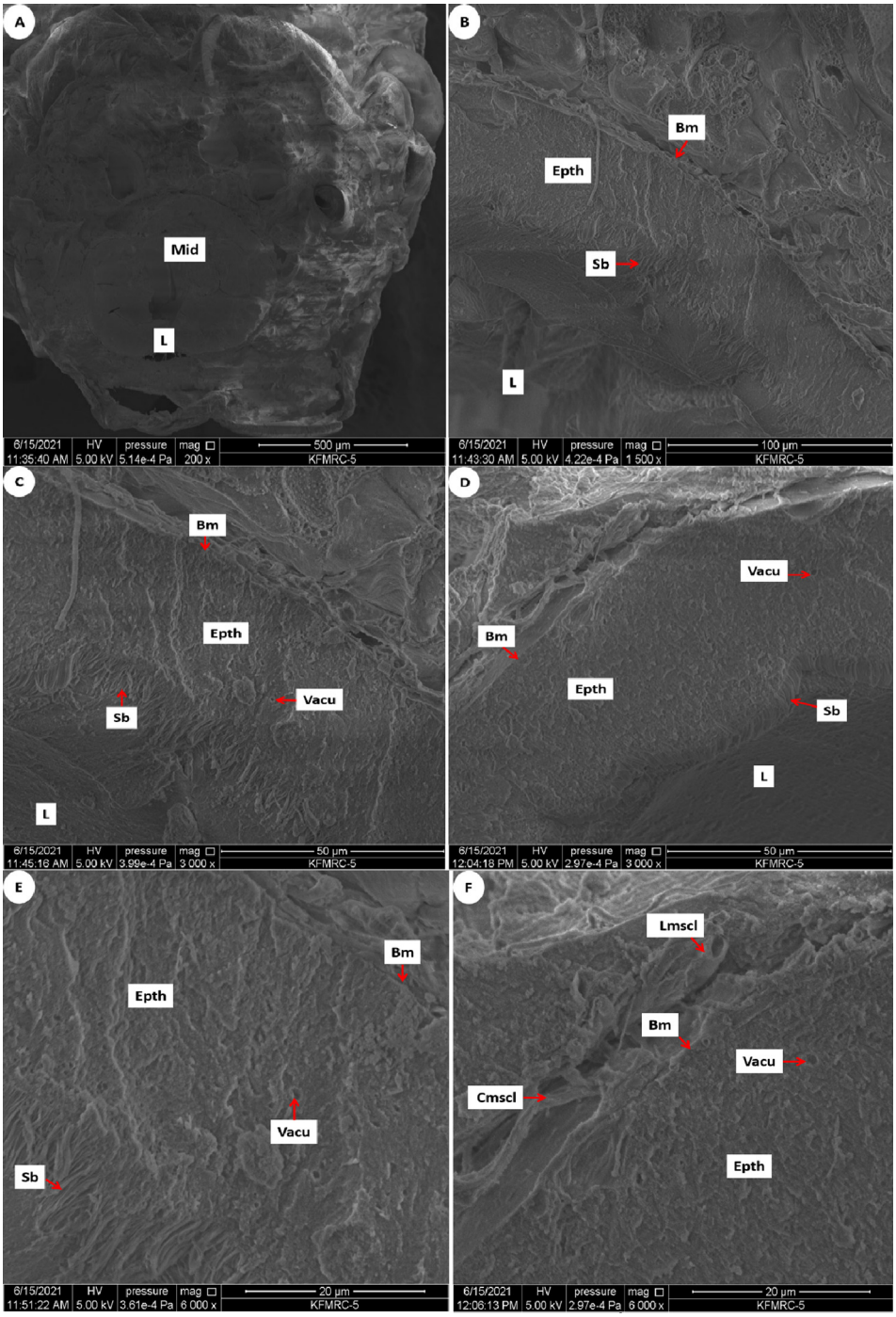

Fig. 7. cross section of the midgut(Mid) in the 5th larval instar honey bee worker after 5 days of Tetracycline group by Scanning Electron Microscope (SEM): A (X –100) B (X-10) C(X- 10)D (X – 20) E(X- 100)F (X- 400); Bm: basement membrane, Cmscl: circular muscles, Epth: Epithelium cells, L:lumen, Lmscl: longitudinal mucles, Sb: striated border., Vacu: vacuoles.

ISCs, resulting in fewer proliferation rates affecting epithelial function and reducing longevity (Forkpah et al., 2014).

The results by Aljedani (Aljedani, 2017)showed the effects of some insecticides on honeybees, Where abamectin impacts midgut cytotoxic cells which may be responsible for digestive midgut problems, epithelial tissue forms when digestion cells die during morphological conditions. The cells are extended into the inner cavity and on the border with the epithelial cell are many holes and curves. This is consistent with what was found in the results of the study by (Dussaubat et al., 2012) where infected bees' epithelial cells showed significant deterioration, which was linked to the downregulation of biological processes such as "positive regulation of cell communication" and "tissue homeostasis and morphogenesis". And in the same case, the digestive cells present several aspects of degeneration, such as great clots of very condensed chromatin and an increase in the size and the types of granules and vacuoles in the cytoplasm (Neves et al., 2003). Gut cells, on the other hand, are normally regenerated through the proliferation and differentiation of stem cells in the basal cell layer, which

then migrates into the lumen once differentiated (Lin and Xi, 2008).

5. Conclusion

It's necessary to know how agrochemicals, such as antibiotics, affect honey bees. As a result, the primary goal of this study was to see how antibiotic treatment (Tetracycline) affected the bio-efficiency of larvae honey bee (*Apis mellifera jemenatica*) workers when they were exposed to various doses of it. These findings indicate that concentration (LC_{50} = 125.25 µg/ml) of antibiotics Tetracycline leads to kills half of the individuals. Tetracycline has a deleterious effect on honeybees, particularly larval honey bee workers, effects on cells that might create digestive problems in midgut, decrease their efficacy and therefore damage the health and vitality of honeybee colonies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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