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Antibacterial Activity of Italian (*Apis mellifera)* bees Venom

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ABSTRACT

The growing resistance of some bacteria to antibiotics as well as the misuse and harmful side effects of antibiotics themselves, emphasizes the need for a more effective and safe alternative. Bee venom as a natural product is a promising alternative to overcome antibiotic resistance and side effects. The objective of this investigation was to determine the efficacy of bee venom collected from pure Italian race of honey bee as antibacterial agent against nine pathogenic bacteria including 6 Gram-positive and 3 Gram-negative strains. The Gram-positive strains were *Staphylococcus aureus, Streptococcus mutans, Streptococcus pyogenes, Lactobacillus caseai, Enterococcus feacalis and Listeria monocytogene.* The Gram-negative strains were *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Bee venom was collected during the summer season of two consecutive years, 2014 and 2015. Bee venom showed potential antibacterial activity against all bacterial strains. The results indicated the possibility of using bee venom as useful alternative to antibiotics against pathogenic bacteria.

KEY WORDS: Italian race, Bee venom, Antibacterial activity, MIC.

1. INTRODUCTION

The microbial resistance to antibiotics and chemicals commonly used against harmful microorganisms has increased (Wright, 2007; Byarugaba, 2009). Bee venom, also called apitoxin, is a colorless liquid secreted by the glands of bees (Hegazi, 2015). Bee venom contains a complex mixture of active peptides, enzymes, and amines (Dotimas and Hider, 1987; Bogdanov, 2015). Bee venom has been used in traditional medicine since ancient times to treat diseases due to its biological activity (Son, 2007). Antimicrobial activity of bee venom against some bacterial strains was previously studied (Harwig, 1995; Koduri, 2002; Hegazi, 2002; 2014; Boutrin, 2008; Park, 2013; Hegazi, 2015). The production and quality of bee venom was found to be affected by many factors including bees' race, bees' age, colony strength, time of collection, feeding, and method of collection (Owen, 1977; Riveros and Gronenberg, 2010). The objective of this study was to evaluate the antibacterial activity of bee venom collected from pure Italian race during the summer season of two consecutive years against some bacterial strains of medical importance.

2. MATERIALS AND METHODS

Venom collection: Bee venom was kindly offered by En. M. El Feel, Department of Apiculture, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza. Venom was collected from the honeybee (*Apis mellifera* L.) workers (Brandeburgo, 1992) of pure Italian race during the 2014 and 2015 summer seasons in the 1st, 2nd, 3rd and 4th week of June. The collection of venom was done using electric shock device (VC-6F model from Apitronic Services, 9611 No.4 Road, Richmond, B.C., Canada). The collection device is composed of collection frame containing wire electrodes installed in parallel to each other. Venom frames were mounted on the top of the wax frames of every hive and they were connected to an electro-stimulator. Electrical current travels through wire electrodes in the form of impulses which stimulated the bees to sting through latex sheet. The latex sheet was placed on a glass plate which served as a collection container of the venom. The odor of the collected venom stimulates other bees to sting through the latex sheet and more venom is collected. The collected dry venom is transferred into special containers using sharp scraper (Fakhim, 1998). The collected venom is transported to the laboratory, packed up in dark glass jars and stored in a cool and dry place.

Bacterial strains: Nine bacterial species including both Gram-positive and Gram-negative were used in this study. The Gram-positive bacteria were represented by *Staphylococcus aureus* (ATCC 25923), *Streptococcus mutans, Streptococcus pyogenes, Lactobacillus caseai, Listeria monocytogene* and *Enterococcus feacalis* while Gram-negative bacteria were represented by *Klebsiella pneumoniae (*ATCC 27736*), Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 27853). The *Streptococcus mutans* and *Lactobacillus casei ss. casei* strains were provided from, Cairo Microbiological Resources Centre (Cairo MIRCEN). The Egypt Microbial Culture Collection (EMCC) number for the *Streptococcus mutans* is 1815^T and for *Lactobacillus casei ss*. casei is 1093^T. The other bacterial strains were kindly provided by the Department of Zoonotic Diseases, National Research Centre.

Antibacterial assay: The bacterial strains were suspended and adjusted by comparison against 0.5 Mc-Farland turbidity standard ($5x10^7$ organisms/ml) tubes. The resulting suspension was further diluted to a final of $5X10^6$ organisms/ml. *Staphylococcus aureus* was cultured on selective agar media enriched with polymyxin according to Finegold and Sweeny (1961), while *Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* were enriched on MacConkey broth. All of the bacterial strains were subcultured on nutrient broth for further bacterial

propagation (Cruickshank, 1979). The broth was inoculated by the 0.20 µl/10 ml broth containing each of the bacterial strains separately, followed by the addition of 40 µl of bee venom in each tube. The tubes were incubated at 37°C for 24 hr. The bacterial growth in all tubes including the control was measured using spectrophotometric assay as turbidity at 420 nm wave length to determine the inhibitory activity of bee venom on different strains. The mean value of inhibition was calculated from triple reading in each test (Hegazi, 2015).

Determination of minimum inhibitory concentrations (MIC): The value of MIC was determined by broth dilution method to serve as quantitative data for the antimicrobial activity of bee venom (Hegazi, 2012; 2015). The bacterial strains were grown in broth media to a mid-logarithmic phase at 1.0×10^6 to 3.0×10^8 CFU/mL. A total of 200 µl of a mid-logarithmic phase culture of bacteria was added to 10 µl of the bee venom range of final concentration; 1-200 µg) in 96 well plate. One well served as a bacterial control contained 200 µl of bacterial inoculates, another well served as negative control contained 200 µl of un inoculated broth media and 10 µl of sterilized distilled water. The plates were incubated at 37ºC for 24h. The inhibition of bacterial growth was determined by measuring the absorbance at 560 nm using ELISA reader. MIC value was measured as the lowest concentration of venom that caused reduction of bacterial growth by more than 90% of the strains.

Statistical analysis: Means and standard deviations of the data collected for each experiment were calculated using Microsoft Excel and statistical significance determined by t-test and one-way ANOVA. Differences in survival were considered significant when $P < 0.05$ (Steel and Torrie, 1980).

3. RESULTS

The weight of bee venom collected in summer season, during the month of June, in two consecutive years 2014 and 2015 was measured in mg/colony as shown in Fig.1. The venom was collected from pure Italian honey bee workers (*Apis mellifera* L.) in the 1st, 2nd, 3rd and 4th weeks of June. The results showed that, although the differences in venom weight between different weeks of collection were not significant, the highest yield as shown by the weight of collected venom was observed in the third week in both years. The lowest weight recorded was (48.9 ± 0.03 mg / colony) while the highest weight recorded was 72.4 ± 1.04 mg / colony.

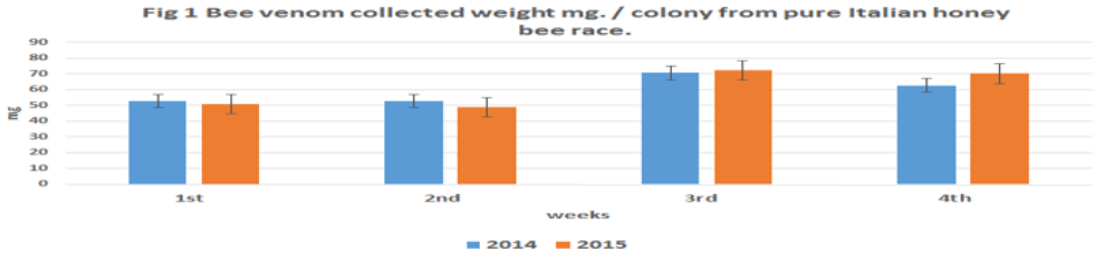

Fig.1. Bee venom Collected weight mg./colony from pure Italian honey bee race

The influence of bee venom collected during the month of June in both years on growth inhibition of Gram-positive (*Staphylococcus aureus, Streptococcus mutans, Streptococcus pyogenes, Lactobacillus caseai, Listeria monocytogene and Enterococcus feacalis)* and Gram-negative bacteria (*Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa*) was shown in Fig.2 and 3. The results showed that there were no significant changes between bee venom collected during 2014 and 2015 on antibacterial activity of different pathogenic bacteria tested.

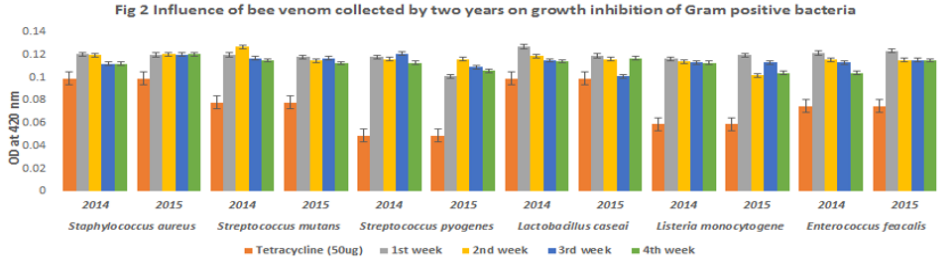

Fig.2. Influence of bee venom collected by two years on growth inhibition of Gram positive bacteria

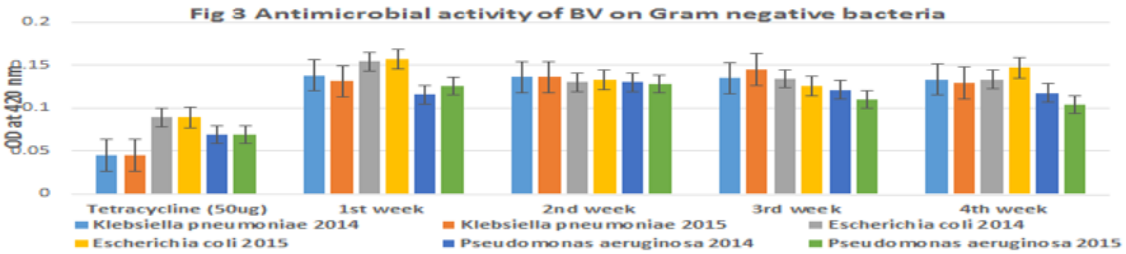

Fig.3. Antimicrobial activity of BV on Gram negative bacteria

The results of MIC of bee venom collected from both years (2014 and 2015) against Gram-positive and Gram-negative bacteria were shown in Figures.4 and 5. The results showed that the differences in MIC between the two years were also not significant.

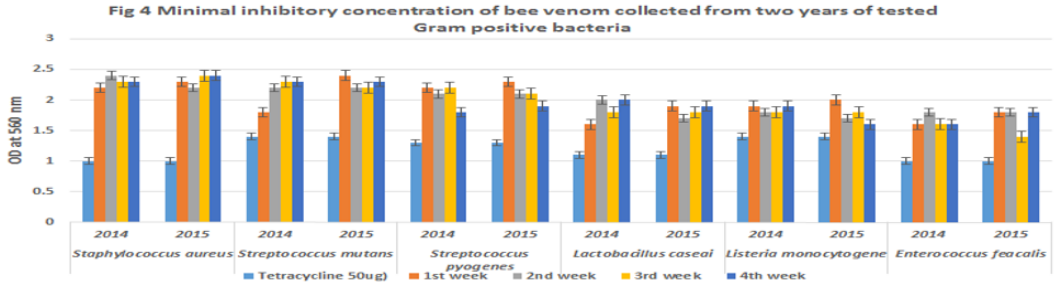

Fig.4. Minimal inhibitory concentration of bee venom collected from two years of tested Gram positive bacteria

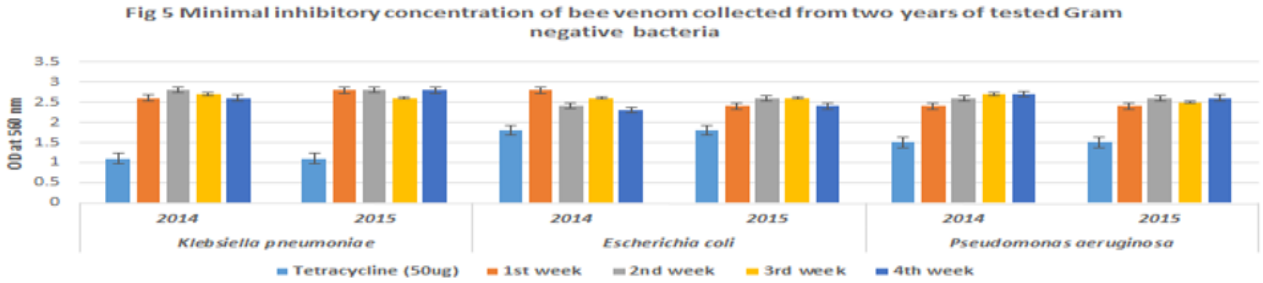

Fig.5. Minimal inhibitory concentration of bee venom collected from two years of tested Gram negative bacteria

DISCUSSION

In the current study, we compared both the yield and antibacterial activity of bee venom collected in the summer season of two consecutive years, 2014 and 2015. The venom was collected on weekly bases during the month of June from pure Italian race honeybee (*Apis mellifera* L.) workers. Spring and summer seasons in general are the best time to collect bee venom when there is a high-level pike in all hive activities (Bachmayer, 1972; Mohanny, 2005; El-Shaarawy, 2007). This might be related to the flowering condition in every season which provides the colony with its requirement of food (nectar and pollen). The yield of venom is also affected by the collection method as shown by Hegazi (2015), when they used two different methods to collect venom from pure and hybrid Carniolan honey bee race. Similar results were also obtained by Leluk (1989), and El-Shaarawy (2007), who showed that bee venom quantity is different from race to the other as well as the ability of bee workers to refill their venom sac after collection.

The antibacterial activity of bee venom collected in two consecutive years (2014 and 2015) against nine pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Lactobacillus caseai*, *Listeria monocytogene*, *Enterococcus feacalis*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*) was investigated. Although the differences in the antibacterial activity of bee venom collected in 2014 and 2015 were not significant, bee venom in general exhibited obvious antibacterial activity against tested strains including Gram-positive and Gram-negative ones. This antibacterial activity may be due to the biological active constituents in bee venom including melittin, apamin, adolapin, mast cell degranulating peptide, enzymes, amines, and non-peptide components (Lariviere and Melzack, 1996; Kwon, 2002). Although it has been reported that bee venom is more effective against Gram-positive than Gram-negative bacteria (Fennel, 1968) a very strong antibacterial activity against *E. coli* has been reported (Stocker and Traynor, 1986; Perumal Samy, 2007; Hegazi, 2014). Several literatures were in contradiction regarding their reports on the efficacy of bee venom against Gram-positive and Gram-negative bacteria. Some publications claimed more antibacterial activity against Gram-positive bacteria while some others claimed more activity against Gram-negative ones (Hegazi, 2002; 2014; 2015; Kondo and Kanai, 1986). One of the important biological activities of bee venom is its ability to form synergistic combination with some antibiotics against resistant bacterial strains. It was reported that a combination of bee venom and kanamycin in the concentration of (8µg/ml) and (10µg/ml) respectively exhibited synergistic activity against a kanamycin resistant strain of *S. aureus* (Benton and Mulfinger, 1989; Rybak, 1994). Han (2007), found that the Korean honey bee venom (KBV) exhibited a potential antibacterial activity against mastitis pathogens including Methicillin-resistant *Staphylococcus aureus* (MRSA). Similarly, Hegazi (2014; 2015), showed that bee venom exhibited potential antibacterial activity against five bacterial strains including *Staphylococcus aureus*, *Streptococcus*

pyogenes, Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa. Sang (2013), found that bee venom can be used as a potential antimicrobial agent against fish pathogenic bacteria.

The minimum inhibitory concentration of the collected venom was measured due to the importance of measuring MIC in evaluating the efficacy of antibacterial agents. This technique has been used previously to evaluate the antibacterial potential of bee venom against several bacterial strains including those causing tooth decay (Leandro, 2015). The values of MIC indicated that bee venom displayed a potential inhibitory activity against both Gram-positive and Gram-negative bacteria.

4. CONCLUSIONS

In the current study, we reported that bee venom collected from the honeybee (*Apis mellifera* L.) workers of pure Italian race during the 2014 and 2015 summer seasons has a promising antibacterial activity against representative strains of both Gram-positive and Gram-negative bacteria. Bee venom warrants further studies to investigate its potential use as a natural alternative to antibiotics against bacterial infection.

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