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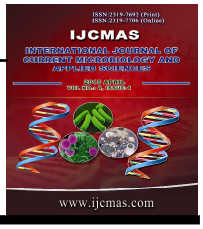

Original Research Article

Antibacterial Activity of Bee Venom Collected from *Apis mellifera* Carniolan Pure and Hybrid Races by Two Collection Methods

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Keywords

Antibacterial Activity, Bee Venom, *Apis mellifera, Carniolan*

A B S T R A C T

The objective of this investigation was to evaluate the antibacterial activity of bee venom from *Apis mellifera* pure Carniolan race as well as its hybrid races collected on latex and fiber sheets against selected Gram-positive and Gram-negative bacterial strains of medical importance. Antibacterial activity of bee venom evaluated against five pathogenic bacterial strains, including *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa*. The results revealed that the amount of bee venom collected from the pure Carniolan race was 46±10.03 mg / colony of venom vz 102 ±11. 91 mg / colony of venom in hybrid. Both bee venom of pure and hybrid bees exhibited antibacterial activity against all five bacterial strains and differs according to the type. Bee venom exhibited antibacterial activity against all five bacterial strains. The minimum inhibitory concentration of BV was determined. These results indicate that BV inhibits the growth and survival of bacterial strains and that BV may be a useful complementary antimicrobial agent against pathogenic bacteria even if bee venom collected by different methods.

Introduction

Bee venom produced by the glands of *Apis mellifera* which has been well documented (Banks and Shipolini, 1986; Ponts et al., 1992 and Palma, et al., 1993). Bee venom is a very complex mixture of active peptides, enzymes, and amines (Habermann, 1972 and Dotimas & Hider, 1987). Many factors affecting honey bee venom production and its quality such as; honey bee race, age of bees, colony strength, season of collection, feeding supplies, race, its defense behavior and method of collection, venom secretion in honey bee worker begins just prior to emergence and increases slowly towards a maximum between the tenth and sixteenth day (Autrum and Kneitz, 1959; Owen et al., 1976). The therapeutic application of bee venom, has been used in traditional medicine to treat diseases (Hegazi, 1998 and 2012). It has biological activity against arthritis (Jae-Dong et al., 2005), rheumatism, pain, rheumatoid arthritis and osteoarthritis (Berman et al., 2000), inhibit mammary carcinoma cell proliferation

(Orsˇolic et al., 2003), cytotoxic to malignant cells both in vitro (Shaposhnikova et al., 2001), cancerous tumors, progressive muscle atrophy (Kim et al., 2000) and skin diseases (Dong et al., 2007). Antimicrobial activity on some Gram-negative bacteria (Harwig et al., 1995, Koduri et al., 2002 and Hegazi et al., 2002 & 2014, Boutrin et al., 2008 and Park et al., 2013). The objective of this investigation was to evaluate the antibacterial activity of bee venom collected by different methods from *Apis mellifera* pure Carniolan race as well as its hybrid against selected Gram-positive and Gram-negative bacterial strains of medical importance.

Materials and Methods

Venom collection:

This work was carried out in the Department of Apiculture, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, during the 2013 summer season. The bee hive as the same power as indicated by many biological activities. Venom collected from the honeybee (*Apis mellifera* L.) workers (Benton et al., 1963 and Brandeburgo, 1992) of pure Carniolan race as well as its hybrid with 1, 2, 3 and 4 week intervals in two experiment of collections by different methods (fiber and latex). The first experiment depends on collecting venom from the top of the frames while the second depend on collecting venom from under the frames with extension collar of a height of 20 mm extent distance between the bottom board and the brood chamber while using collector frame. The bee venom was collected by the electric shock device (VC-6F model from Apitronic Services, 9611 No. 4 Road, Richmond, B.C., Canada), It comprises a bee venom collection frame with wire electrodes installed in parallel to each other. Electrical current goes through them in the form of impulses bee venom frames are mounted on the top or under the frames in every hive and then are connected to an electro-stimulator. Using electrical impulses to stimulate the bee workers to sting through latex or fiber sheet placed on a glass plate and collected the dry venom using sharp scraper (Fakhim Sudan, 1998). Bees that come into contact with the wires received a mild electrical shock and stung onto the glass sheet. The alarm odor, which evaporated from the venom, mobilized and irritated the other bees and they also started to sting. The bee venom collected dries on the glass. The frames with the fresh dried bee venom on them are carefully packed into a special container for transportation to the laboratory. The processing of bee venom is starting right after the frames are brought back in the laboratory. After that bee venom is packed up in the dark glass jars and stored in a cool and dry place

Bacterial strains

Fife bacterial species, including Gram positive and Gram negative were used. These bacteria were kindly provided by the Department of Zoonotic Diseases, National Research Center, Egypt and Department of Botany, Faculty of Sciences, Al Azhar University, Asut Branch, Egypt. The Gram positive bacteria were *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* while the Gram negative bacteria were *Klebsiella pneumonia (*ATCC 27736*), Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35218).

Antibacterial assay

Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Escherichia coli and *Pseudomonas aeruginosa* were used in this study. The bacterial suspension was prepared and

adjusted by comparison against 0.5 Mc-Farland turbidity standard ($5x10^7$ organisms / ml) tubes. It was further diluted to obtain a final of $5X10^6$ organisms / ml. *Staphylococcus aureus* was enriched on polymyxin agar (Finegoldand Sweeny, 1961), as a selective media, while *Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* were enriched on MacConkey broth. All bacteria were subculture on nutrient broth for further bacterial propagation (Cruickshank, et al., 1979). The broth was inoculated by the 0.20 µl/10 ml broth with *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa*, then added 40 µl of bee venom. The tubes were incubated at 37°C for 24 hr. The growth of control bacterial strains as well as inhibition of the bacterial growth due to bee venom was measured by spectrophotometric assay as a turbidity at 420 nm wave length. The mean value of inhibition was calculated from triple reading in each test.

Determination of minimum inhibitory concentrations (MIC)

The MIC were determined by broth dilution method to serve as quantitative data for the antimicrobial (Hegazi et al., 1996, Wu and Hancock, 1999 and Hegazi et al., 2002 Hegazi & Abed Allah, 2012). The bacterial stains were grown in broth media to a mid-logarithmic phase at 1.0×10^6 to 3.0×10^8 CFU/ml. Two hundred microliter of a mid-logarithmic phase culture of bacteria was added to 10 µl of the BV range of final concentration; 1-200 µg) in 96 well plate. One well containing 200 µl of bacterial inoculates served as a bacterial control, while another well containing 200 µl of inoculated broth media and 10 µl of sterilized distilled water were used as a negative control. Culture plates were incubated at 37°C for 24 h. The inhibition of bacterial growth was determined by ELISA reader measuring the absorbance at 560 nm. Results were expressed as MIC, the lowest concentration of the BV that reduces 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).

Results and Discussion

Bee venom collected weight by two methods (fiber and latex) weighted by gm. / colony from pure and hybrid Carniolan honey bee race (Table 1). The results revealed that the overall mean weight was observed in the Hybrid race (102±11.91 and 106±14.66) from fiber and latex respectively.

From the obtained results of Table 2, bee venom revealed to be the most antibacterial activity in all tested samples against different pathogens. The lowest value showed against *Staphylococcus aureus* (0.104 ± 0.005) from venom collected on fiber sheet. While *Streptococcus pyogenes was* (0.125 ± 0.002) from venom collected on latex sheet, *Klebsiella Pneumoniae* lowest value (0.101 ± 0.001) was determined from venom collected on latex sheet. *Pseudomonas aeruginosa* lowest activity was detected (0.143± 0.002) from venom collected on fiber sheet. Meanwhile the lowest activity of bee venom collected from latex sheet was observed against *Escherichia Coli* (0.104 ± 0.009). On the other hand, Tetracycline (50ug) showed effective antimicrobial activity against

different pathogens. It was noticed that the gram negative seemed to be the least sensitive bacteria where gram positive were more affected by tested venoms.

The results of minimum inhibitory concentration and minimum bactericidal concentration of BV were determined. It differs according the bee venom sample (Table 3). The highest MIC of 1st week venom Sample collected on fiber sheet was detected to *Staphylococcus aureus* (1.600 µg/ml), *Streptococcus pyogenes* (1.800 µg/ml) and *Pseudomonas aeruginosa* (1.600 µg/ml). While the highest MIC to *Klebsiella pneumoniae* and *Escherichia Coli* was observed at 2nd week venom Sample collected on fiber sheet (2.000 µg/ml and 2.100 µg/ml) respectively. Tetracycline (50ug) showed variable MIC against different pathogens.

The results obtained showed that bee venom collected every week from the two races colony from top of the frames indicated the pure Carniolan race was lower in amount of collected venom (46 ± 10.03 ml. gm. / colony), while hybrid race (75 ± 17.47 ml. gm. / colony) these results are in agreement with the finding of Bachmayer et al., (1972); Mohanny (2005) and El-Shaarawy et al., (2007). They mentioned that in general spring and summer seasons are the best seasons to collect bee venom when there is a pike in all hive activities, also there is a relation with the flowering condition of the season that provided the colony need for food (nectar and pollen).

Bee venom collected from under the frames with extension collar of a height of 20 mm. showed the lower amount of bee venom recorded by pure race (102 ± 11.91 ml. gm. / colony) while it was 106±14.66 ml. gm. / colony with hybrid race. This result confirms those obtained by leluk et al., (1989); El- Ashhab, (2001) and El-Shaarawy et al., (2007) who stated that bee venom quantity had a relationship with bee strain that control the amount of venom from strain to another and the ability of bee worker to fill bakes the venom sac for what taken before the next collection time.

Results of Table 2, revealed that the lowest value showed against *Staphylococcus aureus* (0.104 ± 0.005) from venom collected on fiber sheet. While *Streptococcus pyogenes was* (0.125 ± 0.002) from venom collected on latex sheet, *Klebsiella Pneumoniae* lowest value (0.101 ± 0.001) was determined from venom collected on latex sheet. *Pseudomonas aeruginosa* lowest activity was detected (0.143± 0.002) from venom collected on fiber sheet. Meanwhile the lowest activity of bee venom collected from latex sheet was observed against *Escherichia Coli* (0.104 ± 0.009). The antimicrobial activity of bee venom due to several peptides like melittin, apamin, adolapin, mast cell degranulating peptide, enzymes, biologically active amines, and non-peptide components (Lariviere and Melzack, 1996; Kwon et al., 2002). Fennel et al. (1968) reported that the venom contains mellitin which is active against gram-positive more than gram-negative bacteria. In contrast, a stronger activity on *E. coli* had been reported previously for bee venom (Stocker and Traynor, 1986; Perumal Samy et al., 2007 and Hegazi et al., 2014). The study provides evidence that bee venom has antibacterial effective against both Gram positive and Gram-negative bacteria. These results are in general agreement with those found by Hegazi et al., (2002 and 2014) which showed that bee products were less effective against *E. coli*. Kondo and Kanai (1986) who found that mycobacteria and staphylococci were affected by the bee venom fraction (melittin), but not *E. coli.* Also, Benton and Mulfinger (1989) reported

that bee venom (8µg/ml) and kanamycin (10µg/ml) exhibited synergistic activity against a kanamycin resistant strain of *S. aureus*; 4-10; mean 6.6 µg/ml (Rybak et al., 1994). The relative sensitivities of the bacteria were qualitatively estimated by measuring the zones of inhibition. Ortel and Markwrdt (1955) quantitatively determined the zones of inhibition. They found that Gram positive organisms were more sensitive at lower concentrations of bee venom than Gram negative. Sang et al., (2013) studded, bee venom isolated from honey bees *Apis mellifera* and assessed for its potential use as an antimicrobial agent against fish pathogenic bacteria. They found that bee venom exhibited antibacterial activity against all tested infectious fish pathogens. Han et al., (2007) found that that the KBV has a potential antibacterial effect against mastitis pathogens. The highest inhibitory zones of 21.4 mm were observed from *Staph. aureus*. It was followed by MRSA at 21.2 mm against the standard of 10.8 mm. The KBV exhibited the maximum inhibitory. Hegazi et al. (2014) found that the bee venom (BV) isolated from bees (*Apis mellifera*) was assessed for its potential use as an antimicrobial agent against five pathogenic bacteria. It exhibited antibacterial activity against all five bacterial strains.

The lowest value showed against *Staphylococcus aureus* (0.104 ± 0.005) from venom collected on fiber sheet. While *Streptococcus pyogenes was* (0.125 ± 0.002) from venom collected on latex sheet, *Klebsiella Pneumoniae* lowest value (0.101 ± 0.001) was determined from venom collected on latex sheet. *Pseudomonas aeruginosa* lowest activity was detected (0.143± 0.002) from venom collected on fiber sheet. Meanwhile the lowest activity of bee venom collected from latex sheet was observed against *Escherichia Coli* (0.104 ± 0.009). On the other hand, it was noticed that the gram negative seemed to be the least sensitive bacteria where Gram positive were more affected by tested venoms. These findings confirmed by many authors as Bachmayer et al., (1972); leluk et al., (1989); El- Ashhab (2001); Mohanny (2005); El-Shaarawy et al., (2007) (Han et al., (2007). and Hegazi et al. (2014).

Table.1 Weight of bee venom (gm./colony) from pure and hybrid Carniolan honey bee race

Treatment	*Apis mellifera*			
	Carniolan race		Hybrid race	
	Fiber	Latex	Fiber	Latex
1st week Sample	52	89	82	74
2nd week Sample	32	41	84	100
3rd week Sample	72	111	132	145
4th week Sample	29	42	111	105
Over all mean	46±10.03	75±17.47	102±11.91	106±14.66

Table.2 Influence of bee venom collected by two different methods (fiber and latex) on growth inhibition of different bacteria

Treatment	*Staphylococcus aureus*		*Streptococcus pyogenes*		*Klebsiella Pneumoniae*		*Pseudomonas aeruginosa*		*Escherichia coli*	
	Fiber	Latex	Fiber	Latex	Fiber	Latex	Fiber	Latex	Fiber	Latex
Normal growth	1.550 ± 0.005	1.550 ± 0.005	1.400 ± 0.001	1.400 ± 0.001	0.900 ± 0.150	0.900 ± 0.15	1.700 ± 0.150	1.70 ± 0.150	1.550 ± 0.005	1.55 ± 0.005
Tetracycline (50ug)	0.095 ± 0.001	0.095 ±0.001	0.057 ± 0.002	0.057 ± 0.002	0.349 ± 0.001	0.349 ± 0.001	0.069 ± 0.004	0.069 ± 0.004	0.049 ± 0.003	0.049 ± 0.003
1st week Sample	0.104± 0.005	0.155 ± 0.005	0.500± 0.008	0.125 ± 0.002	0.178± 0.003	0.101 ± 0.001	0.197± 0.0025	0.167± 0.001	0.173± 0.004	0.114 ± 0.009
2nd week Sample	0.155 ± 0.005	0.305 ± 0.005	0.257± 0.002	0.325 ± 0.002	0.106 ± 0.001	0.116 ± 0.001	0.187± 0.001	0.267± 0.001	0.114 ± 0.009	0.134 ± 0.009
3rd week Sample	0.352± 0.002	0.552± 0.002	0.3117± 0.002	0.3117± 0.002	0.201± 0.001	0.109 ± 0.001	0.201± 0.009	0.401± 0.009	0.510 ± 0.012	0.510 ± 0.012
4th week Sample	0.404± 0.005	0.115 ± 0.005	0.615± 0.008	0.257± 0.002	0.173± 0.003	0.106 ± 0.001	0.143± 0.002	0.267± 0.001	0.273± 0.004	0.104 ± 0.009

Table.3 Influence of bee venom collected by two different methods (fiber and latex) on minimal inhibitory concentration

Treatment	*Staphylococcus aureus*		*Streptococcus pyogenes*		*Klebsiella Pneumoniae*		*Pseudomonas aeruginosa*		*Escherichia coli*	
	Fiber	Latex	Fiber	Latex	Fiber	Latex	Fiber	Latex	Fiber	Latex
Tetracycline (50ug)	1.000	1.000	1.600	1.600	1.800	1.800	1.200	1.200	4.400	4.400
1st week Sample	1.600	2.800	1.800	2.400	2.200	2.200	1.600	1.900	2.100	2.200
2nd week Sample	2.800	2.800	2.400	2.600	2.000	2.400	2.600	1.700	2.800	2.400
3rd week Sample	3.600	3.200	3.600	3.600	2.400	2.500	1.800	1.800	3.800	3.800
4th week Sample	2.600	2.800	2.300	2.400	2.700	2.600	2.600	1.900	2.400	2.600

The results of minimum inhibitory concentration and minimum bactericidal concentration of BV were determined. It differs according the bee venom sample (Table 3). The results of minimum inhibitory concentration and minimum bactericidal concentration of BV were determined. It differs according the bee venom sample (Table 3). The highest MIC of 1st week venom Sample collected on fiber sheet was detected to *Staphylococcus aureus* (1.600 µg/ml), *Streptococcus pyogenes* (1.800 µg/ml) and *Pseudomonas aeruginosa* (1.600 µg/ml). While the highest MIC to *Klebsiella pneumoniae* and *Escherichia Coli* was observed at 2nd week venom Sample collected on fiber sheet (2.000 µg/ml and 2.100 µg/ml) respectively. Tetracycline (50ug) showed variable MIC against different pathogens. These results confirmed previously by some researchers who reported that antibacterial effect to Gram-negative and Gram-positive bacteria differs among antibacterial agents (Monk et al., 1996. it was evident from the study that the KBV has indeed an antibacterial effect against both Gram-negative and Gram positive bacteria (Han et al., 2007). Antimicrobial activities were not different statistically. It was clearly demonstrated that the honey bee venom inhibited the growth of seventeen Gram positive bacteria strains and two Gram negative strains isolated from bovine mastitis in Korea (Park et al., 2013). The minimum inhibitory concentration of BV was determined by Hegazi et al., (2014).

They found that BV inhibits the growth and survival of bacterial strains and that BV may be a useful complementary antimicrobial agent against pathogenic bacteria.

It could conclude that from these results indicate that BV inhibits the growth and survival of bacterial strains and that BV may be a useful complementary antimicrobial agent against pathogenic bacteria. These experimental data confirm the previous work that bee venom can inhibit bacterial growth even when collected bee venom with two different methods.

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