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THE INHIBITORY EFFECT OF HONEY BEE VENOM AGAINST SOME DIFFERENT MICROOGANISMS

El-Gizawy, S.A.
Dept. Agric. Microbiol., Faculty of Agric., Cairo Univ., Giza, Egypt.

ABSTRACT

Venom collected from honey bee (*Apis mellifera L.*) workers of Egyptian, Carniolian and Carnionlian hybrid races were microbiologically examined for their inhibitory effect against different microorganisms by using disc diffusion technique. There was a clear inhibitory effect (IE) for all bee venom types and this (IE) was strongly dependent on venom type and the type of test microorganism. Bee venom from the Egyptian race showed the strongest inhibitory effect (13.3-23.7 mm) against microorganisms while, the venom obtained from Carniolian race gave the lowest effect (10.3-16.3 mm). Bee venom from Carniolian hybrid race revealed a moderate inhibitory action (11.7-19.0 mm). Gram-positive bacteria as well as yeast and moulds demonstrated the most susceptible cells for venom type while, in contrary Gram-negative bacteria manifested quite tolerance for the antimicrobial action of bee-venom. Antimicrobial activity of bee venom proved to be concentration-dependent and it had a bacteriostatic effect at low concentrations while, at higher concentrations it considered as bactericidal. The incorporation of bee venom in growth medium negatively affected the progress in growth behavior of microorganisms and this effect increased with increasing venom concentration. Viability of *Listeria monocytogenes* and *E.coli* cells in their growth media had been lost and completely died-off after 24 and 48 hrs of incubation in the presence of high venom concentration (1000 µg/ml) respectively. The presence of a moderate venom concentration (400 µg/ml) in the medium delayed the bacterial growth and finally cells disappeared after five days of exposure. Providing growth media with low venom concentrations considerably suppressed the growth of the two bacterial strains. This phenomenon can be used in medical purposes and drug preparations or by some modifications in food preservation.

Keywords: Bee venom, antimicrobial effect, microorganisms, inhibitory effect

INTRODUCTION

Bee venom is considered an important bee product recently used in apitherapy. It is collected from acidic and alkaline glands associated with the sting apparatus of honey bee workers. Bee venom reaches its maximum secretion in worker bees sixteen days old and ceases after 20 days (Owen and Bridges, 1976). Bee venom is a colorless liquid that can be changed into crystalline by drying and the color of crystallized form ranges from white to brownish yellow (Piek, 1986). Some factors may affect bee venom production, e.g., race, age of bees, season, type of feeding and finally the defense behavior of the bee (Marz *et al.*, 1981; Omar, 1997). Amounts of venom differed between each bee species and ranged between 27 and 218 µg/bee (Schmidt, 1995). Pure and whole dried bee venom usually used in drug preparations (Simics, 1999). It could be suggested that race and age of bees play an important role in the amount of bee venom produced by honey bee colonies (Nour *et al.*, 2004). Bee venom contains a number of peptides, enzymes and amines responsible for inflammation and antimicrobial activity (Inoue *et al.*, 1987; Omar, 1997 and Nour *et al.*, 2004). There are several

sources of variability in quantity and structure of the venom including age of bees, season of the year, location of bees as well as race or hybrid (Marz *et al.*, 1981; Inoue *et al.*, 1987; Omar, 1994; Robinson and Otis, 1996). There was clear difference between age of bees among the tested honey bee races and hybrids in number of bee venom protein bands and molecular weights (Nour *et al.*, 2004). It had been concluded by some authors (Hussein, 2000 and Saleh, 2001) that bee venom types had clear inhibitory action against microorganisms and this action was more pronounced against Gram-positive than Gram -ve cells. They added that *Staph. aureus* cells were the most susceptible cells towards bee venom types while, Gram-negative organisms showed some tolerance. Nowadays, more attention is given to collect histories and information about bee venom therapy, together with medical uses of other bee products (Garedew *et al.*, 2004; Torres *et al.*, 2004). A new trend in bee venom application related to the use of bee-stinging in the treatment of many diseases had been mentioned by Al-Anssary (2003). In a recent study related to the effect of honey bee and wasp venoms against pathogenic bacteria, Mohammed and Zakaria (2005) concluded that the growth of bacteria had been affected by the presence of venoms and Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) were more sensitive to both venoms than Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*). They also added that *E. coli* cells were the most resistant to wasp venom.

The aim of the present investigation is to determine the extended inhibitory effect of bee venom against different microorganisms, to study the nature of this action in relation to venom concentration and finally, to follow the growth behavior of two selected bacterial strains (*Listeria monocytogenes* & *Escherichia coli*) in medium supported with different bee venom concentrations.

MATERIALS AND METHODS

Source of the bee venom races

Three samples of bee venom (Egyptian race, Carniolian race and Carniolian hybrid) were collected from the apiary of the Faculty of Agric. in Giza during spring (2005). Not less than one hundred individuals from worker bees were used for venom collection. Bee venom of each race and hybrid was collected from venom sacs dissected under water to avoid evaporation of the volatile compounds according to the method of Pence (1981). Tubes containing bee venom were tightly closed and wrapped with aluminum- foil before keeping in refrigerator until the time of use.

Preparation of aqueous extracts of venom types

Five-milliliters of sterilized distilled water were added to 5 mg of each venom type and shaked for 1-2 min to extract the active components in water. This suspension was considered as stock solution that contained 1000 µg of active components of bee venom per milliliter. Discs of filter paper (Whatman no.1) of 5 mm diameter were immersed in the stock solutions and placed on the top of agar-plate according to the method of disc diffusion technique (Norrell, 1990). Zones of inhibition were measured (in mm) including disc diameter.

Preparation of different bee venom concentrations

Different aqueous venom concentrations represented 50, 100, 200, 400 and 500 µg/ml were prepared from the Egyptian race venom and each concentration was used for immerging the discs and completion of the disc diffusion technique.

Microorganisms

Eleven microbial strains representing four Gram +ve bacteria (*Bacillus cereus, Staphylococcus aureus, Micrococcus* sp. and *Listeria monocytogenes*); four Gram –ve bacteria (*Escherichia coli, Enterobacter aerogenes, Salmonella typhimurium* and *Pseudomonas aeruginosa*) ; one yeast (*Saccharomyces cerevisiae*) as well as two fungi (*Aspergillus niger and A. oryzae*) obtained from the culture collection of the Microbiological Dep., Faculty of Agric., Cairo Univ., were used as test organisms to determine the inhibitory action of bee venom by disc diffusion technique. Two selected bacterial strains (*Listeria monocytogenes and E. coli*) were only used to perform growth behavior in media contained variable bee venom concentrations in order to follow the growth behavior of each bacterial strain in its growth media containing different venom concentrations.

Media

Nutrient agar slants or plates were used in maintaining, sub-culturing or performance of the disc diffusion while, malt agar slants were used in case of yeast or mould growth as well as mould spore production. Fungal spores were yielded by scraping from the old slant agar surfaces in tubes of sterilized distilled water (contained few drops of Tween 80 to keep spores suspended in water). Spore suspension was filtered through strain-cloths and its density was adjusted to 10^6/ml. Nutrient broth was used in the case of following the growth of bacterial strains in the media supplemented with bee venom concentrations.

Growth behavior of the test organism

The growth behavior of each of the two bacterial strains (*L. monocytogenes and E. coli*) was followed in nutrient broth medium provided with different bee venom concentrations (100, 400 and 1000 µg/ml). Initial numbers of the test organism (10^3 cfu/ml) were added and numbers of bacterial species were estimated after 6, 12, 24, 36, 72, 96 and 120 hrs of incubation at 30 °C for the first microorganism or 37 °C for the second organism.

Statistical analysis

Each treatment was conducted in three replicates. All the experiments were arranged in a completely randomized design, L.S.D. test was employed for mean comparisons according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

It is clear from data in Table (1) that all types of bee venom had inhibitory effect against various microorganisms ranged between 10.33 and 24 mm as measured by the diameter of inhibition zone. Bee venom of the

Egyptian race revealed the strongest inhibitory effect against test microorganisms as compared with the other types while, Carniolian race showed the lowest inhibitory effect of bee venom. Carniolian hybrid race of bee venom had moderate inhibitory activities against the test microorganisms.

Data in Table (1) also indicate that Gram $+^{ve}$ microorganisms were generally more susceptible to the venom than Gram $-^{ve}$ members inside a particular venom type. *Micrococcus* sp., *Staph. aureus* and *Sacch. cerevisiae* were the most susceptible strains among the test organisms while, *Ps. aeruginosa*, *Sal. typhimurium* and *E. coli* had some tolerance to the tested bee venom.

Table (1): Values of inhibition zone of bee venom types against different selected microorganisms (mm)

Strain type	Inhibition zone of bee venom types (mm)			
	Egyptian race venom	Carniolian race venom	Carniolian hybrid venom	Mean of strain
B. cereus	20.67 c	15.00 g-i	16.67 ef	17.44 C'
Staph. aureus	22.00 bc	15.33 f-h	18.00 de	18.44 AB'
E. coli	15.67 f-h	11.33 mn	13.67 i-k	13.56 D'
Sal. typhymurium	14.67 h-j	12.67 k-m	13.67 i-k	13.67 D'
Ps. aeruginosa	13.33 jk	10.33 n	11.67 l-n	11.78 E'
Micrococcus sp.	23.67 a	13.00 kl	19.00 d	18.56 AB'
L. monocytogenes	21.33 bc	15.33 f-h	18.00 de	18.22 A-C'
Ent. aerogenes	15.67 f-h	11.67 l-n	13.67 i-k	13.67 D'
Sacch. cerevisiae	22.33 ab	16.33 fg	18.00 de	18.89 A
Asp. niger	21.67 bc	15.00 g-i	16.67 ef	17.78 BC'
Asp. oryzae	22.00 bc	15.00 g-i	16.67 ef	17.89 BC'
Type mean	19.36 A	13.73 C	15.97 B	

Means followed by the same letter(s) are not significantly different from each other at 0.5

As a conclusion, the inhibitory action of bee venom against microorganisms is significantly influenced by the venom type as well as type of microorganism. Yeast and mould strains, similarly, to bacteria were affected by the bee venom and placed in the most susceptible category.

Different aqueous concentrations of venom (Egyptian race) ranged from 1000 to 50 µg/ml were examined and the inhibitory zones of such concentrations were determined by the disc diffusion technique with selected bacterial strains. Data in Table (2) show values of inhibition zones (mm) of the test organisms in relation to venom type and its concentration.

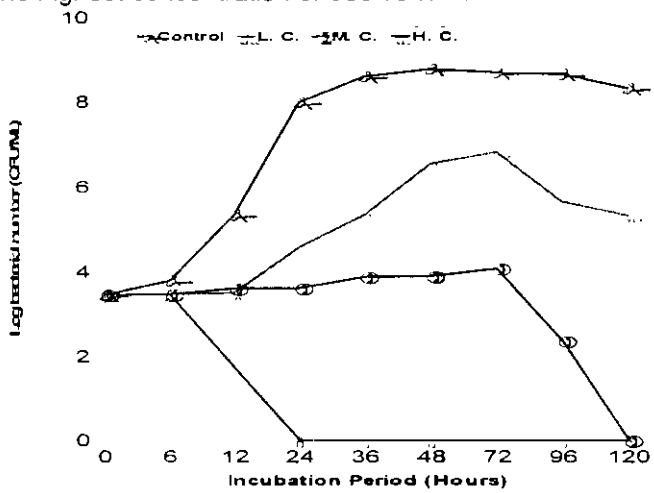
Data in Table (2) assured again the stronger inhibitory action of bee venom from Egyptian race against bacterial strains as compared to that of Carniolian race type. It is also concluded that antibacterial action of the venom is concentration-dependent where, the antimicrobial activity increased with increasing venom concentration. Data in the table again revealed the high susceptibility of Gram +ve organisms to bee venom as compared to G-ve cells. The lowest concentration (50 µg/ml) in use showed the minimum inhibition zone of microorganisms and that value may be considered as the value of minimum inhibitory concentration for the bee venom.

Table (2): Effect of venom type and venom concentration on the inhibitory action against some selected bacterial strains

Bacterial strains	Inhibition zones of the venom concentrations (mm)											
	Egyptian race venom						Carniolian race venom					
	50	100	200	400	500	1000	50	100	200	400	500	1000
B. cereus	6.33 X-Z	10.00 P-R	14.67 F-G	17.00 E	18.00 DE	20.67 BC	5.33 Z	8.00 T-V	9.67 Q-S	12.33 I-L	13.33 HI	15.00 F
Staph. aureus	6.67 W-Y	9.67 Q-S	12.67 H-K	15.67 F	17.67 DE	22.00 A	6.00 X-Z	7.67 U-W	10.33 O-Q	12.67 H-K	13.33 HI	15.67 F
L.monocytogenes	7.67 U-W	11.00 M-P	14.67 F-G	18.33 D	19.67 C	21.33 AB	6.33 X-Z	8.00 T-V	9.67 Q-S	12.67 H-K	13.33 HI	15.33 F
E. coli	6.67 W-Y	8.67 S-U	11.33 L-O	12.33 I-L	13.67 GH	15.67 F	5.67 YZ	7.00 V-X	8.67 S-U	10.00 P-R	11.33 L-O	11.67 K-N
Sal. typhimurium	6.33 X-Z	8.00 T-V	9.67 Q-S	11.33 L-O	13.00 H-J	14.67 FG	6.33 X-Z	8.33 TU	10.67 N-Q	11.00 M-P	11.67 K-N	12.67 H-K
Ps. aeruginosa	6.00 X-Z	7.00 V-X	10.00 P-R	12.00 J-M	12.67 H-K	13.33 HI	5.33 Z	6.67 WY	8.67 S-U	8.33 TU	9.00 R-T	10.33 O-Q

Means followed by the same letter(s) are not significantly different from each other at 0.5
Values of venom concentrations are expressed as (µg/ml)

Behavior of two selected bacterial strains (one Gram-positive and the other Gram-negative) were followed in nutrient broth provided with different bee venom concentrations. Fig (1) shows the growth of *Listeria monocytogenes* cells in broth medium supplemented with bee venom in various concentrations. The growth of *List. monocytogenes* cells was greatly affected with high concentrations (400 and 1000 µg/ml) of the venom while, it showed a moderate effect at low concentration (100 µg/ml). *L. monocytogenes* cells completely died off after five days of incubation in the presence of 400 µg/ml of medium while, cells disappeared only after one day of exposure to the highest concentration of bee venom.



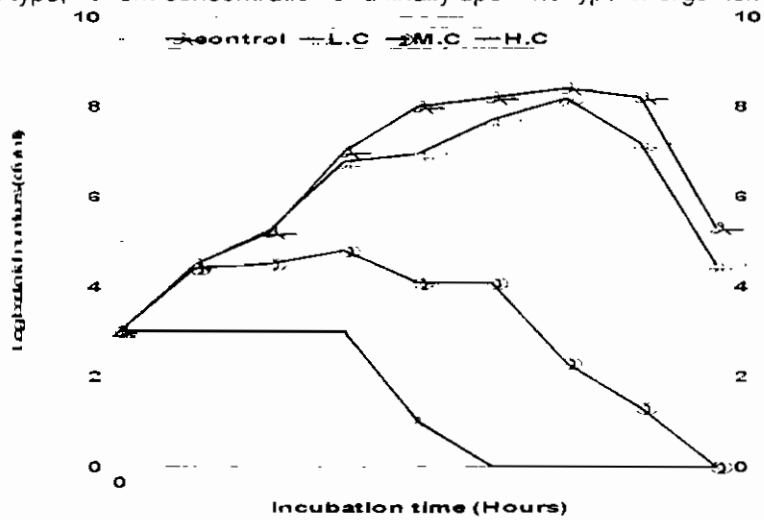
L.C., Low concentration (100 µg/ml)
M.C., Medium concentration (400 µg/ml)
H.C., High concentration (1000 µg/ml)

Fig. (1): Behavior of *Listeria monocytogenes* cells in nutrient broth media contained different venom concentrations at 30 °C.

Data in the figure strongly announced that inhibitory action of the venom is concentration-dependent.

The growth patterns of *E.coli* cells in nutrient broth provided with different bee venom concentrations are demonstrated in Fig. (2). As seen with *L. monocytogenes* , cells of *E. coli* showed an inhibition in growth behavior and this inhibition increased with increasing venom concentration. Cells of *E. coli* completely disappeared after four days and only one day of incubation at 400 and 1000 µg/ml, respectively. Cells of *E.coli* showed a slight tolerance against bee venom than *L. monocytogenes* do at all concentrations.

As a general conclusion, bee venom had an antimicrobial action against almost all types of microorganisms and this action is dependent upon venom type, venom concentration and finally upon the type of organism.



L.C., Low concentration (100 µg/ml)
M.C., Medium concentration (400 µg/ml)
H.C., High concentration (1000 µg/ml)

Fig. (2): Behavior of *Escherichia coli* cells in nutrient broth media contained different venom concentrations at 37 °C.

Results in this investigation proved that bee venom collected from different bee types had an inhibitory effect against all test microorganisms and that activity is concentration-dependent. The inhibitory action of bee venom can be considered bacteriostatic at low concentrations while, it demonstrated a bactericidal effect at high concentrations. The ability of bee venom to inhibit microorganisms was more announced against G $^{+ve}$ organisms as compared to G –ve cells. These data agree with the findings of some authors (Hussein, 2000 and Saleh, 2001). They added that most susceptible strains to bee venom were *Corynebacterium pseudotuberculosis* followed by *Staph. aureus* and *Streptococcus faecalis* as measured by inhibition zones. The previous authors also indicated that most susceptible strains among G- bacteria were *Aeromonas hydrophila* followed by *Sal. typhimurium* and *E. coli*.

Antimicrobial activity of bee venom has primary been referred to the action of peptides mainly melittin-peptide and this compound is responsible for pore formation in the cytoplasmic membrane of both G^+ and G- ve organisms, this compound is a non-cell-selective cytolysin (Beven and Wroblewski, 1997; Matsuzaki, 1997; Oren and Shai, 1997). Subbalakshmi *et al.* (1999) mentioned that these peptides (melittin) from bee venom exhibited potential antibacterial activity towards both G^+ and G-ve bacteria. It had been suggested that variations in quantity and composition of bee venom may be referred to bee race (pure race or hybrid) and age, location of bee, season of the year and to the quality of nutrition in bee colonies (Marz *et al.*, 1981; Inoue *et al.*, 1987; Omar, 1994; Robinson and Otis, 1996). It is most likely that potency of bee venom against microorganisms is largely dependent on bee venom protein bands and its molecular weights (Nour *et al.*, 2004). The chemical composition of venom protein is consequently dependent on bee race or hybrid as well as other pre-mentioned factors.

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التأثير التثبيطى لسم نحل العسل ضد بعض الميكروبات المختلفة

سمير الجيزاوى
قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة القاهرة

أجريت هذه الدراسة لمعرفة التأثير المثبط لسم النحل على مختلف الميكروبات (بكتريا- خميرة- فطريات)- وتم قياس التأثير المثبط لسم النحل على الميكروبات باتباع تكنيك الانتشار من القرص الورقى المشبع بمحلول السم فى مزرعة ميكروبية غزيرة النمو. استخدم لذلك ثلاثة أنواع من سم النحل تختلف باختلاف فصيلة النحل الذى أخذ منها (بلدى نقى، كرينولىنقى، كرينولىهجين) على احدى عشر سلالة ميكروبية- وقد تمثلت النتائج المتحصل عليها فيما يلى:

- أظهرت كل أنواع سم نحل العسل تأثيرا مضادا لنشاط جميع الميكروبات (بكتريا-خميرة-فطر) من خلال ظهور منطقة تثبيط حول القرص المبلل، وكان أقوىتأثير مضاد للميكروبات ناتج من سم النحل البلدى النقى (١٣،٣-٢٣،٧ مم) يليه فى التأثير سم النحل من سلالة هجين كرينولى (١١،٧-١٩،٠مم).
- كان السم الناتج من سلالة النحل كرينولى النقى هو الأقل فى القوة المضادة للميكروبات فقد أعطى منطقة تثبيط تعادل (١٠،٣-١٦،٣مم).
- أظهرت الميكروبات اختلافات جوهرية فى مدى تأثرها بسم نحل العسل، حيث كانت البكتريا الموجبة لصبغة جرام أكثر حساسية للسم - بينما أظهرت البكتريا السالبة لجرام بعض المقاومة للفعل المضاد، كما أن الخميرة (خميرة الخباز) وبعض الفطريات أظهرت حساسية كبيرة لسم النحل.
- امكن استنتاج أن تأثير سم النحل على نمو الميكروبات فى الوسط الغذائى يعتمد بشكل كبير على تركيزه - ففى حالة التركيزات المنخفضة منه كان تأثيره مثبط (عائق) للنمو البكتيرى **bacteriostatic** ، بينما فى حالة وجود تركيزات عالية نسبيا منه كان تأثيره قاتل للبكتريا **bactericidal** .
- أظهرت النتائج أن النشاط المضاد للميكروبات لسم النحل يعتمد فى تأثيره على كل من نوع الميكروب بالاضافة الى نوع النحل المنتج للسم.
- أوضحت النتائج المتحصل عليها أن الفعل التثبيطى لسم النحل يعتمد بدرجة كبيرة على تركيزه فى الوسط حيث قل الفعل التثبيطى للسم مع زيادة التخفيف ضد كل من البكتريا الموجبة والسالبة لصبغة جرام على السواء حتى أن أقل تركيز من سم النحل (٥٠ ميكروجرام/مل) أعطى أقل منطقة تثبيط بكتيرى - مما يمكننا من اعتبار هذا التركيز هو "أقل تركيز مثبط" من سم النحل.
- وجد أن إمداد بيئة النمو البكتيرى بتركيزات مختلفة من السم أدى الى إختلاف فى سلوك النمو البكتيرى سواء مع خلايا الليستريا مونوسيتوجينس أو مع خلايا اشيريشيا كولاى حيث أن نمو الميكروبين فى وجود تركيزات عالية نسبيا من السم أدى الى موت وعدم ظهور خلايا حية فى المزرعة ذات تركيز ١٠٠٠ ميكروجرام/ مل بعد يوم واحد ، يومين من التحضين لكل من ميكروب الليستريا واشيريشيا على التوالى- بينما على تركيزات متوسطة (٤٠٠ ميكروجرام/مل) ماتت نفس الخلايا بعد خمسة أيام لكل ميكروب من الميكروبين على حده.
- أظهرت التركيزات البسيطة من السم (١٠٠ ميكروجرام/مل) فى بيئة النمو تثبيط ملحوظ فى شكل منحنى النمو لكل ميكروب- مقارنة بالكنترول.
- نظرا للتأثير القوى لسم نحل العسل ضد الميكروبات المختلفة، فانه يمكن الاستفادة من ذلك فى علاج الكثير من الأمراض ذات الأصل الميكروبى إما عن طريق التعرض مباشرة لمحاليل ذات تركيزات عالية من السم فى حالة الأمراض الجلدية (التى سببها فطريات ، خمائر ، بكتريا) او فى معالجة الجروح- كما يمكن الاستفادة من الفعل التثبيطى لسم نحل العسل فى العلاجات الصيدلية عن طريق تضمين الدواء لنسب من السم على إنفراد أو بالاشتراك مع مضادات حيوية أخرى من أجل زيادة الفاعلية المضادة للميكروبات، أيضايستخدم سم النحل الآن فى علاج الكثير من الأمراض المتنوعة عن طريق التعرض لعدد معين من لسعات نحل العسل ، كما يجب البحث عن طريقة مناسبة للاستفادة من سم نحل العسل فى الصناعات الغذائية كمادة حافظة (غير سامة) من أصل طبيعي.