CONTRIBUTIONS TO THE ANTIMICROBIAL ACTION OF BEE VENOM

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Bee venom is a substance whose biologically active components, represented by: the A and B phospholipides, melitin, apamin, hialuronidase, peptides and other elements, cause a series of reactions when introduced into the body, making bee venom usable in treating various diseases.

P. KOMAROW, A. ERSHTEIN, I. KOOP, N.P. IOIRIS and others have demonstrated that a 1/50000 solution of bee venom in products is sterile. It was also proved that the *paramecium* (a unicellular organism in infusoria class) is immediately dissolved in the 1/10000 bee venom solution and in 30 seconds at a concentration of 1/50000. A concentration of 1/500000-1/600000 will stimulate the *paramecium* multiplication. Therefore, bee venom has various biological actions depending on the concentration degree of the solution.

DEREVICI and DIMA (1969) when experimenting the effect of bee venom inoculations into cultures of epithelial cells of monkey kidney, have noticed important morphophysiological changes in these. Many studies were carried out that pointed to the inhibitory action of bee venom on bacteria and fungi development. Thus, the experiments of several researchers carried out on acetonic extracts from integral sac bee venom have pointed out their bacteriostatical action upon certain microorganisms like: *Microbacterium phlei*, choleric vibiro and Eberth bacillus. ARTEMOW and coll. (1967), after an inoculation of freeze dried bee venom preparations in nutritive media, inseminated and incubated at 37 ^{O}C for 7 days, have proved the antimicrobial action of bee venom.

Mention should be made that the further studies carried out by many researchers and by us have demonstrated that all microorganisms have a different behaviour against the bee venom. Considering these multiple effects of bee venom and the results of the researches, the aim of our paper was to study the behaviour of certain microorganisms subjected to its action.

With this aim in view the study had two objectives:

1. Verification on the sterility state of pure bee venom in study:
2. Bee venom action on several pathogenic and pathogenic conditioned bacteria.

Material and Methods

We have used the following materials: haemolys tubes, usual and selective culture media, test microorganisms (*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella typhy, Pseudomonas aeruginosa, Bacillus cereus, Bacillus brevis, Bacillus orpheus, Bacillus larvae*) and the solution for analysis (1% solution in distilled water).

To accomplish the first objective we have proceeded to the insemination of a watery solution of bee venom 1% (5ml) in media no. 1 and no. 2 to check sterility (40 ml). Incubation was then carried out at 37 ^{O}C for 48 and 72 hours for bacteria and at 28 ^{O}C for 5-6 days for fungi. The results were interpreted at the end of the time periods. Table 1 shows the preliminary results obtained.

Table 1

Control on the Sterility of Bee Venom

Inseminated medium	Microbiological results
Medium no. 1	sterile
Medium no. 2	fungi belonging to *Penicillium genus*

The test tubes containing the no. 1 inseminated medium have maintained the initial transparency throughout the incubation period. In the test tubes containing the no. 2 inseminated medium notice was made of the changes occurred in the transparency of the medium on the 3rd and 6th day after insemination. Whereas in the solid medium no. 2, we have noticed the appearance of some fungi colonies belonging to the *Penicillium* genus (table 1).

In order to accomplish the second proposed objective, we have previously verified the antibacterial action using the difusion-metrical method - the technics of washers.

After an incubation at 37 ^{O}C for 24 hours, we have measured the diameter of the inhibitory zone of culture development around the washers, according to the classical method. The preliminary results on the bactericidal action of bee venom in 1% solution are presented in table 2.

Table 2

Antibacterial Action of Bee Venom

Crt. No.	Microorganisms	Diameter of the inhibitory zone (mm)
1.	*Staphylococcus aureus*	12
2.	*Streptococcus pyogenes*	9
3.	*Salmonella typhy,*	9
4.	*Escherichia coli*	8
5.	*Klebsiella pneumoniae*	5
6.	*Pseudomonas aeruginosa*	5
7.	*Bacillus cereus*	16.4
8.	*Bacillus brevis*	18.5
9.	*Bacillus orpheus*	15
10.	*Bacillus larvae*	13

We have noticed that in solid media in plates, the bee venom solution had an evident bactericidal action, represented through large inhibitory zones, whose diameters were beyond 18 mm.

In order to establish the inhibitory bacteriostatical and bactericidal doses, we have applied the dilutions method, composed of the following stages, as imposed by the working technology:

2.1. Insemination in liquid medium depending on the bacterial strain for test and thermostation at 37 ^{O}C for 24 hours.

2.2. Preparation of the testing series:

We have distributed 1 ml culture medium of suitable liquid into 8 tubes and 1 control tube. The solution for test was poured into tube 1 and the 8 dilutions were carried out in asepsis conditions. The respective series of dilutions were: ½, ¼, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256. Tube no. 9 was used as control into which the test strain grew. Each tube including the control one received a drop of the bacterial suspension. The results were interpreted after 24 hours of incubation at 37 ^{O}C.

2.3. Insemination in plates containing solid medium from the tubes where no bacterial growth was noticed and results interpretation after 48 hours of thermostation.

Results and Discussion

Here below we give the results of the researches carried out on 10 bacterial species and bee venom solutions at suitable concentrations at whose level was noticed the bacteriostatical and bactericidal effect, as presented in table 3. In *Staphylococcus aureus* the bacteriostatical effect occurred in 1/128 dilution and the corresponding concentration to the 0.000078 g/ml dilution when the culture medium turned quite turbid into the tube. The 1/64 dilution and the concentration of 0.00015 g/ml marked the bactericidal effect. Up to this dilution the culture media from the corresponding plates at dilutions: ½, ¼, 1/8, 1/16, 1/32 showed no microbial growth.

The inhibition of the development and multiplication of *Streptococcus pyogenes* occurred in 1/64 dilution and concentration of 0.00015 g/ml. Complete destruction of germs occurred at the 1/32 dilution and 0.00031 g/ml concentration. A conclusion may be drawn that the two microorganisms were sensitive to quite low concentrations.

As shown in the table, the bee venom had significant antibacterial effects on bacilli negative gram. Thus, for *Escherichia coli* the bacteriostatical effect occurred in 1/32 dilution and concentration of 0.00031 g/ml. The destructive action occurred at the dilution of 1/16 and concentration on 0.00062 g/ml. *Salmonella typhimurium* had a similar behaviour.

In our tests, *Kleibsiella pneumoniac* showed a lower sensitivity. The bacteriostatical action took place at the 1/16 dilution and concentration of 0.00062 g/ml, and the bactericidal effect at the 1/8 dilution and concentration of 0.0012 g/ml.

It's worth mentioning that *Pseudomonas aeruginosa* too belonging to *Pseudomonas* genus showed a low sensitivity to the action of bee venom. Therefore, the multiplication process was affected at the concentration of 0.0012 g/ml and 1/8 dilution and the bactericidal effect occurred at 0.0025 g/ml concentration with the corresponding ¼ dilution.

The results of researches on the study of the action of bee venom on certain sporulated aerobic bacteria are the following: the bacteriostatical effect for *Bacillus cereus* occurred in 1/32 dilution and corresponding concentration of 0.00031 g/ml and the bactericidal effect at 1/16 dilution and concentration of 0.00062 g/ml. For *Bacillus bravis* the bacteriostatical effect of bee venom occurred in 1/64 dilution and concentration of 0.00015 g/ml and the bactericidal effect in 1/32 dilution and concentration of 0.00031 g/ml. As regards *Bacillus orpheus* the bacteriostatical action took place at 1/16 dilution and concentration of 0.00062 g/ml and the bactericidal action at 1/8 dilution and concentration of 0.0012 g/ml.

Table 3

Bacteriostatical and Bactericidal Effect of the Bee Venom

Dilution	Concentrat-ion g/ml	*Staphi aureus*	*Strept. pyogenes*	*Escher. coli*	*Salmonella typhi*	*Klebsiella pn.*	*Pseud. aeruginoasa*	*Bacillus cereus*	*Bacillus brevis*	*Bacillus orpheus*	*Bacillus larvae*
1/2	0.01	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile
1/4	0.0025	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile **	Sterile	Sterile	Sterile	Sterile **
1/8	0.0012	Sterile	Sterile	Sterile	Sterile	Sterile **	Isolated colonies *	Sterile	Sterile	Sterile **	Isolated colonies *
1/16	0.00062	Sterile	Sterile	Sterile **	Sterile **	Isolated colonies *	Growth **	Sterile **	Sterile	Isolated colonies *	Growth
1/32	0.00031	Sterile	Sterile **	Isolated colonies *	Isolated colonies *	Growth	Growth	Isolated colonies *	Sterile **	Growth	Growth
1/64	0.00015	Sterile **	Isolated colonies *	Growth	Growth	Growth	Growth	Growth	Isolated colonies *	Growth	Growth
1/120	0.000078	Isolated colonies *	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth
1/256	0.000039	Growth like the control ∞	Growth like the control ∞	Growth like the control ∞	Growth like the control ∞	Growth like the control ∞	Growth like the control ∞	Growth like the control ∞	Growth like the control ∞	Growth like the control ∞	Growth like the control ∞
Control		∞	∞	∞	∞	∞	∞	∞	∞	∞	∞

** = bacteriostatical effect; ** = bactericidal effect*

Bacillus larvae, when subjected to the action of bee venom, was inhibated in its multiplication at 1/8 dilution and concentration of 0.0012 g/ml and the bactericidal effect took place in ¼ dilution and concentration of 0.0025 g/ml.

Our studies show that a higher sensitivity to the action of bee venom had *Staphylococcus aureus*, *Streptococcus pyogenes* followed by *Escherichia coli*, *Salmonela typhi*, *Bacillus brevis*, *Bacillus cereus*. A lower sensitivity was noticed in *Klebsiella pn*., *Bacillus orpheus*, *Pseudomonas aeruginosa* and *Bacillus larvae*.

Both present researches and the following ones open new prospects to making a wider theraputical use of bee venom under various pharmacological forms.

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