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# The seasonal activity of hyaluronidase in *venom* of a honey bee (*Apis mellifera* L. *caucasica*) in various regions of Azerbaijan

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**Abstract**
Activity of hyaluronidase in venom of a honey bee *Apis mellifera* L. *caucasica* from various regions of Azerbaijan was studied. The highest mean activity of hyaluronidase was in the venom collected in Zaqatala (103.1 IU/mg), the lowest activity was in the venom, collected in the surrounding the city of Baku (69, 5 IU/mg). The activity of hyaluronidase in venom was determined depending on the seasons of the year. The highest enzyme activity in the venom collected in all regions was established in summer and lowest in autumn. The results of the experimental data can be used for determining the biological activity and quality of bee venom for the manufacture of drugs based on apitoxin.

**Keywords:** *Apis mellifera L. Caucasica***,** hyaluronidase, venom of honey bee, apitoxin

## 1. Introduction
Beekeeping in Azerbaijan is one of the most environmentally friendly types of farming. At a time when food safety issues are of global importance, this special attention is given to development of beekeeping in the world.
The venom produced by honey bee (*Apis mellifera* L. *caucasica*), -is one of the first objects of zoological and toxicological research. The venom of bees is very different from all the other poisons. Thus, snake venom is anticoagulant, while the bee venom is a coagulant; from cobra venom it differs by the presence of the diffusion factors [1].
Deciphering the chemical composition of bee venom is largely due to his understanding of its harmful effect. Biologically active substances included in the bee venom, are divided into several groups. The first of them - are proteins with enzymatic properties, among which the most important are phospholipase $A_2$, hyaluronidase and acid phosphatase. The next group consists of toxic polypeptides: mellitin, apamin, MSD- peptide tertiapine, secapine. The third group includes biogenic amines [2-4].
Hyaluronidase – enzyme, hydrolyzing viscous hyaluronic acid, which helps keep the body tissue cells together. With the destruction of hyaluronic acid, intervals between the cells lose their viscosity, which facilitates the penetration of other venom components. Thus, hyaluronidase facilitates the penetration of bee venom into the body. For these reasons, some hyaluronidase preparations have been used successfully in medicine for the resorption of scars, scars in the healing of wounds and burns of the skin and mucosal surfaces, as the scars are formed by connective tissue [5].
Hyaluronidase of bee venom, in its properties, is similar to the enzymes from other sources, however, the optimum pH for it lies in the more acidic range (pH 4-5). Hyaluronidase of bee venom - glycoprotein is composed of mannose, galactose and fucose at a ratio of 4: 1: 1. Carbohydrate subunit is linked to a protein N - glycoside bridge through asparagine and N - acetylglucosamine. This is one of the high polymeric components of the venom. The molecular weight of enzyme is 35000-53000 [6, 7].
The composition of the venom is complex. The sequence of formation of venom components, the quantity and quality of biological adaptation reflect bee family elements to the environment. The various components of the venom have not the same safety and resistance to various influences.
Thus, thermolabile hyaluronidase loses its activity when it is heated. Stability is also low and it decreases when stored under normal conditions.

The ecological status of the regions of Azerbaijan ambiguously. All progressive pollution of biosphere of Azerbaijan by man caused emissions of the industrial enterprises, leads to catastrophic worsening of an ecological situation in the nature, imbalance of natural processes, destruction of the ecosystems leading to considerable saturation by toxic elements, including heavy metals.

The problem of pollution of biosphere of Apsheron of Azerbaijan by toxic, heavy metals like Pb, Hg, As, Cd, Ni, Cu, Va, Zn, Co, Mo, Sr and other metals, has arisen by technogen emissions of the industrial enterprises in an atmosphere. Since the biological activity of the venom is made up of the activities of its constituent parts, it is of great interest to study the activity of hyaluronidase from different regions of Azerbaijan.

The aim is to study the seasonal activity of hyaluronidase in the venom of the honey bee (*Apis mellifera* L. *Caucasica*) from a variety of environmental indicators of Azerbaijan's regions.

## 2. Materials and Methods

The material of the research were the venom of the honey bee samples collected on apiaries environs of Baku, Sumgait, Shamakhi, Sheki, Balakan, Zaqatala. Collection of venom was done during in spring, summer, autumn.

In order to determine the activity of hyaluronidase, 0.01g of venom was taken and was dissolved in 10 ml of water. 0.1 ml of the resulting solution and 0.2 l of acetate buffer solution was placed in two tubes and 0.3 ml of acetate buffer solution was added into the third tube (control solution). The tubes were placed in an incubator at temperature 37C, through 5min 0.2 mL of 0.2% hyaluronic acid solution were added into all tubes sequentially and kept thermostated for 15 minutes.

Then sequentially with 30 seconds interval, potassium kalium tetraborate calculated by number titer of 0.8 mole was added into the tubes and placed in a water bath at a temperature of 100C for 3 minutes, then the test tubes was transferred in an ice bath for 10 min. In the cooled tube was added 3 ml of Erlich reagent diluted and placed in an incubator at a temperature of 37C for 20 minutes.

Into the cooled tube 3 ml of Erlich reagent diluted was added and it was placed in an incubator at a temperature of 37 $^0$C for 20 minutes. The tubes were then cooled to room temperature and immediately measured value of the optical density of the resulting solutions with a spectrophotometer CENESYS 10S UV-VIS, at a wavelength of 586 nm.

The optical density value of the working standard solution sample glucosamine was measured parallelly, which for titer calculated according to the number of moles of potassium tetroborata 0.8 moles was added to the diluted 0.5 ml glucosamine, placed in a water bath at a temperature of 100 $^0$C for 3 minutes and then received as described above [5, 8].

Enzyme activity was expressed as the hyaluronidase mME. Statistical processing of experimental data was performed by Lakin [9].

### 2.1 The purpose of research

The aim of the work is to study the effect of the degree of contamination of the biosphere Azerbaijan by heavy metals on the venom and study the seasonal activity of hyaluronidase in the venom of the honey bee (*Apis mellifera* L. *Caucasica*) from a variety of environmental indicators of Azerbaijan's regions.

## 3. Results and Discussions

Ecological problems of preserving the habitat of bees, leads to the inevitable loss of bee products. The material was collected from different regions of Azerbaijan. Beside with ecologically clean area - Sheki, Shamakhi, Zaqatala and Balakan suitable climate and rich honey land, there are also areas (Baku, Sumgayit), contaminated by oil, oil products, insecticides and heavy metals. Therefore, quality indicators of venom and other waste products of the honey bee differ.

The highest average annual activity of hyaluronidase was found in the venom collected in Zaqatala and was 103.1 mIU. The lowest average enzyme activity was registered in Baku (69.5 mIU). The difference between the activity of hyaluronidase toxins collected in the apiaries Zakatally and Baku is 33.6 mIU (Table 1). Annual mean values of hyaluronidase activity in Balakan, Shamakhi, Sheki and Sumgayit are respectively 95.5 mIU, 102.4 mIU, 92.3 mIU and 81.0 mIU.

In regions with similar climatic conditions hyaluronidase activity differs slightly. Comparative analysis of the activity of hyaluronidase in the venom of bees of different habitats showed that activity in the forest and foothills - Sheki, Shamakhi, Balaken, and Zakatallah is high and in low-lying areas - Baku, Sumgait is minimum.

Thus, on the basis of the data on the activity of the enzyme studied, we can conclude that the venom collected from Zakatall, Balakan and Shamakhi has high biological activity.

**Table 1:** The average annual activity of hyaluronidase in the venom of the honey bee (*Apis mellifera* L. *Caucasica*) from various regions of Azerbaijan

| Regions of Azerbaijan | The activity of enzyme, mIU |
|---|---|
| Baku | 69.5 ±2.8 |
| Sumgayit | 81.0±2.3 |
| Zaqatala | 103.1 ±15.5 |
| Balakan | 99.5±0.8 |
| Shamakhi | 102.4±1.5 |
| Sheki | 92.3 ±0.8 |

The activity of hyaluronidase in the venom of the honey bee from various regions of Azerbaijan, depending on seasons of year of collecting venom was defined. Change of activity of hyaluronidase is indicated in the diagram (Fig.1).

Analyzing the indicators of activity of hyaluronidase in the venom of bees of different regions on the seasons of the year, it was revealed that in the summer months, the enzyme activity was higher than in the other seasons of the year. In Zaqatala, value of enzyme activity in the summer months was higher than in other regions - 105.0 mIU, in the spring months the highest figure was 103.0 mIU, and in the fall - 101.2 mIU.

On apiaries of Baku the measure of the activity of hyaluronidase in the venom of bees was the lowest in summer (71.1 mIU) in spring-70.3 mIU, and in the fall -67.0 mIU. İt is known that. Toxic gland and storage tank of summer bees are better developed than in the spring and autumn, so in the summer months, bee venom has the highest activity.

It is therefore logical that we have obtained figures hyaluronidase enzyme activity in the venom of bees and reached its highest value in the summer months.

The diagram shows that the difference in the activity of hyaluronidase in the venom collected in Baku ın summer and autumn is 3.1 MIU (R<0.1), summer and spring 0.8 mIU (R>0, 5) and in spring and autumn is 2.3 mIU (R<0.1). All changes in activity are statistically unreliable.

In Sumgayit the activity of enzyme in the venom, collected in the summer is 2.1 mIU more than in the venom, collected in autumn (R<0.2) and 1mME more than in the venom collected ın spring (R>0.5). The difference between the activity of an enzyme in the venom of the spring and fall is 1.1 MIU (R<0.5). These changes in activity were also not statistically significant.

In Zagatalla actıivity of enzyme in the summer is 3.6 mIU more than in autumn (R<0.05) 2 mIU more than in spring (R<0.2). The difference between the activity of enzyme in autumn and spring is 1.8 MIU (R<0.5).

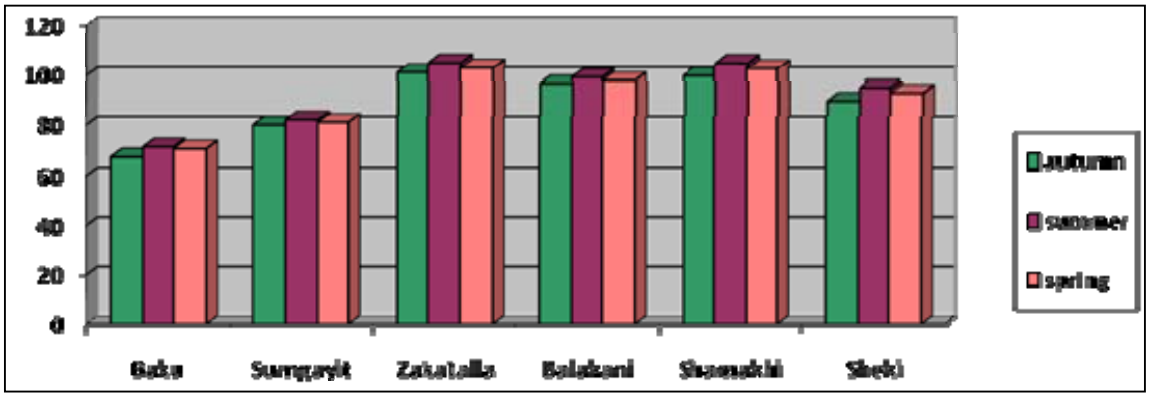


**Fig 1:** Activity of hyaluronidase in the venom of the honey bee (*Apis mellifera* L. *Caucasica*) depending on the season of the year from various regions of Azerbaijan (mIU)

In Balaken, the difference between activity of the enzyme in the summer and autumnsis 2.7 MIU (R<0.1), between summer and spring is 1.4 mIU (R<0.5), between spring and autumn is 1.3 mIU (R<0.5).

The activity of enzyme in the venom, collected in Shamakhi, in the summer is 5.1 mIU higher than in autumn (R<0.001) and 2.1 mIU higher than in spring (R<0.1). The difference between the enzyme activity in spring and autumn is 3 mIU (R<0.1).

Activity of hyaluronidase in the venom, collected in Sheki, in the summer is 5.1 mIU more than in autumn (R<0.001) and 2,1mME more than in spring (R<0.1). The difference between the enzyme activity in spring and autumn is 3.2 MIU (R<0.1).

Thus, the maximum activity of the enzyme hyaluronidase in the venom of the honey bee, assembled in all investigated areas was established in summer, and the lowest - in autumn. However, the difference in the activity of the enzyme is not statistically significant. Only in the venom, assembled in Shamakhi and Sheki in the summer of hyaluronidase activity was significantly greater than activity in autumn (R<0.001).

Among the areas studied in all seasons the highest enzyme activity was established in Zaqatala, and the lowest in Baku.

Taking into account the increase in the production of medicines based on bee venom. steady demand for raw venom can be expected. In determining the activity of the venom the activity of most important components of venom: melittin, phospholipase $A_2$, hyaluronidase is determined.

These components play an active role in the overall effect of venom. İn the composition of bee venom, all ingredients have strict specialization and at the same time act synergistically, complementing and reinforcing each other.

Toxic activity of hyaluronidase is negligible, except for allergenic properties, as the inherent macromolecular foreign agents. However, for the manifestation of the overall effect of the venom, specified appointment of an enzyme in venom is indispensable. Therefore venom collected from Zaqatala, Balakan, Shamakhi has high biological activity.

Isolation and purification of the ingredients of bee venom have significantly expanded the range of their practical use as tools for biological research and in the manufacture of drugs.

## 4. Conclusions

In this way bee products are harmless, non-toxic, increases the protective forces of the living organism against negative influences of the environment, have a fallout, adaptive, termoprotector effect, help eliminate toxins from the body.

The results of the experimental data can be used in assessment of the quality and biological activity of honeybee venom in the manufacture of pharmaceuticals based on Apitoxins.

From the research implies that was is studied hyaluronidase activity in venom of a honey bee *Apis mellifera* L. *caucasica* from different regions of Azerbaijan

The highest mean activity of hyaluronidase in the venom collected in Zaqatala in the summer.

The lowest activity in the venom, collected in the surrounding the city of Baku in the autumn.

In regions hyaluronidase activity in the venom of bees of different habitats showed that activity in the forest and foothills – Sheki (92.3 ±0.8 IU/mg), Shamakhi (102.4±1.5 IU/mg), Balakan (99.5±0.8 IU/mg), and Zakatallah (103.1 ±15.5 IU/mg) is high and in low-lying areas - Baku (69.5±2.8 IU/mg), Sumgait (81.0±2.3 IU/mg) is minimum.

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