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STUDY OF BIOLOGICALLY ACTIVE PARTICULARITIES OF PHEROMONE OF ANXIETY (THE SECRET) OF THE RED HYBRID WORM OF CALIFORNIA

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Abstract

The objective of the study consisted in determining some biologically active properties of the pheromone of anxiety(secret), obtained from the tissues of the Red Hybrid Worm of California for the purpose of its use in veterinary medicine. The object of the research served: the Red Hybrid worm of California, the pheromone of anxiety both natively and in various dilutions (from 1:1 to 1:100). During the study, the following methods were used: to obtain the pheromone of anxiety and sterilizing of it; the determination of bactericidal activity and lysozyme was performed by the photonefelometry method. As a result of the research it was found that the pheromone of anxiety manifested bactericidal activity towards microbial cultures Staphylococcus aureus (№ 209), Escherichia coli, Micrococcus luteus (Micrococcus lysodeikticus), Candida albicans depending on the used dilutions. In the undiluted native secret, the bactiricidal activity, tested by using these crops, constituted respectively 552.00%, 355.00% and 13,500.00%, and in the dilutions 1:1 - 1:100 it oscillated respectively from 250.00%, 370.00% and 3,900.00% till 23.00%, 42.00% and 100.0%. Towards the microbian culture Candida albicans microbial activity was not found. The lysozyme activity of the undiluted pheromone of anxiety constituted 10.20%, and in the dilutions 1:10 - 1:100 it oscillated from 8.60% to 8.70%, which led to the fact that research would no longer be continued. Thus, it was found that the pheromone of anxiety, obtained from tissues of Red Hybrid worm of California, has manifested a bactericidal activity depending on the concentration and type of microbial cultures on which the test was performed.

Key words: *bactercidal activity, lysozyme activity, anxiety pheromone, preparation, the Red Hybrid Worm of California*

INTRODUCTION

Since ancient times, worms have been a biological research object. The worms of the Red Hybrid of California (Eisenia foetida andrei) used as biotransformers of organic waste can also be used as proteinaceous additives in the foodration of poultry, pigs, fish and other animals. Also the biological mass of earthworms can be used in the pharmaceutical industry, medicine and other branches of the national economy.

The biological mass of worm contains essential quantities of ferments, vitamins, micro- and macro-elements and other biologically active substances [7]. From one tonne of organic waste, in the process of their bioconversion by the use of the technology of worm cultivation, is obtained 70-100 kg of biological mass of worms, in which are contained 67-72% of the protein, including amino acids: methionine, lysine, cystine and the others. Also, the biological mass of worms contains up to 12-17% of lipids, including phospholipids, saturated and unsaturated fatty acids.

According to the investigations it was found that on the surface of one hectare of the worm cultivator from the biological mass of earthworms, can be obtainedup to 40 tons of protein flour, which according to its protein content, not only does not yield, but to a certain extent exceeds meat flour, fish, soybeans and fodder yeasts. In nature, a more advantageous source is not known, which under industrial conditions would produce such a valuable protein for the livestock sector.

The characterization of food value of protein flour, obtained from the biological mass of worms, as compared to that of another origin, is exposed in the work of A.M. Igonin [9].

The biological mass of worms and products derived from it are widely used in the livestock sector in many countries: China, Japan, Kyrgyzstan, Ukraine, Russia, and so on. The amount of protein required for the body of the animals can be replaced by using the biological mass of worm's. Thus, in China and Japan, to broiler poultry were added to the food ration during the three weeks, 5-6 worms for each individual, and in India, in the diet of piglets daily, 25-30 of living worms. In Stavropol, adding 1.00% of live earthworms to the duck diet contributed to theincreaseof egg productivity with 25.00% [9].

The biological mass of worms in the form of flour can be used in feed of aquarium fish, reptiles, expensive fur animals and others [6].

During the last decades of the twentieth century, the biologically active substances of worm tissues, and especially the Red Hybrid of California, began to be used for the purpose of preparing the substances for use in veterinary and human medicine [8, 10].

In the works of B. M Anohin and others [2], [3], are exposed the preventive results of the use of extract obtained from the biomass of worms in the treatment of chemical burns. V.Abramov and others [1] evaluated the role of the biologically active peculiarities of preparations obtained from tissues of worm of the Red Hybrid of California on rabbit body resistance and immune stimulatory actions at salmonella of rabbits. In works L.Cremeneac and others [6], is mentioned the importance of the use of extracts obtained from worms tissues on immune, clinical and physiological status at young cattle.

The preparation obtained from the biological mass of worm of the Red Hybrid of California after sterilization was used for treat of wounds that were not prone to scarring, skin diseases and other aetiologies. This preparation also possesses immunomodulatory activity and antitumor action.

In order to obtain biologically active substances from tissues of worms Red Hybrid of California was elaborated the method to obtain of the preparation "Aflegmin", which has been tested for the treatment of skin burns and eye irritationcaused by chemical substances, conjunctivitis and irritation [4, 5].

Iu.Holodova and others [8] mentioned that the biological mass of earthworms can be used as: a protein complex with a content of 56% which contains a number of amino acids needed to increase the body mass of the animals; lipid complex with a content of 10% lipid complex, recommended as an active addition to cosmetic preparations and lipid-based amino acid concentrate - 10%, an aqueous solution used for the treatment of eye diseases.

Preventive positive results have also been obtained when using the preparation of earthworms in the treatment of such maladies: mastopathy, malignant tumors, psoriasis and osteochondrosis [8].

In the Scientific and Practical Institute of Biotechnologies in Animal Husbandry and Veterinary Medicine, has also been carried out research to obtain preparations from tissue of worms Red Hybrid of California and have been studied some particularities biologically active of them for the purpose of using medicinal remedies for veterinary medicine. One of the preparations was the pheromone of anxiety (the secret).

As a result of the research of the biologically active characteristics of the obtained preparation, it was found that it does not possess toxic and allergic activities, but it shows the bactericidal activity towards some microbial cultures.

MATERIALS AND METHODS

The research was carried out under the conditions of the laboratory "Ecological problems in zootechnics", within the Practical Institute of Biotechnologies in Animal Husbandry and Veterinary Medicine. All the works were performed in sterile conditions.

The research objects were: the worms the Red Hybrid of California, three fractions (concentrated, transparent, the precipitate kept in various periods) of the pheromone of the anxiety (the secret) obtained from the worm's tissues in native state as well as in various dilutions (1:1, 1:5, 1:10, 1:20, 1:50 and

1:100); Microbial cultures of Staphylococcus aureus, Escherichia coli, Micrococcus luteus (Micrococcus lisodeikticus), Candida albicans.

During the determination of the biologically active features of the pheromone of anxiety and its fractions, the following methods were used: for obtaining the pheromone of anxiety and its fractions, which included two steps: the first - the preparation of earthworms to impulse stimulation by electrocution and the second - the creation of sterile maximum conditions for obtaining the pheromone of anxiety and its fractions with the subsequent study of their biologically active features.

All works were done in sterile conditions. At the beginning of the research, the box where the works were carried out was disinfected using the bactericidal lamp.

Determination of bactericidal activity and lysozyme was performed by method of photocolorimetry using the KFK-2 photocolorimeter. As tests were used microbial cultures Staphylococcus aureus, Escherichia coli, Micrococcus luteus, Candida albicans.

Initially was obtained a one day culture of the microbial cultures mentioned above. Then in the test tube was placed 1.0 ml native secret or fractions of it were added 2.5 ml of the nutrient environment of agar of meat and peptone. To the same tube was added one drop of the day microbial culture for testing.

The contents of the test tube were divided into two equal parts by placing them in 2 sterile test tubes, so samples of the experimental variant were prepared. In the blank control test tubes were taken: 2.5 ml of the nutrient environment of meat and peptone and one drop of the microbial culture used for testing, in which the volume was also divided into two equal parts.

Subsequently, the content of a one test tube from an experimental variant and one of the control were calorimetric, using photocolorimeter KFK-2 with a green shake and the cuvette of 3.0 mm. Colorimetry was performed in comparison with distilled water. The other two experimental and control test tubes, with sterile media, were placed in the thermostat for 3 hours, then subjected to colorimetry just as the previous samples.The results of the colorimerization were used to calculate the difference between experimental and control samples. Calculations were performed according to formula (1), thus bactericidal activity (AB) was determined:

$$AB = 1- (\frac{A}{B} - \frac{a}{b}) \times 100\% \quad \textbf{(1),}$$ where

AB - bactericidal activity;

A - extension of the experimental sample, after 3 hours of storage in the thermostat;

B - extension of the control sample, after 3 hours of storage in the thermostat;

a - the initial extension of the experimental sample;

b - the initial extension of the control sample;

100 - transformation of the coefficient in%.

The activity of lysozyme was determined in the sterile anxious pheromone as Micrococcus luteus microbial culture was used as test. The lysozyme activity was determined by the photocolorimetric method.

The research was carried out on both in the pheromone of native anxiety and its dilutions 1:1; 1:5 1:10; 1:20; 1:50 and 1:100. Also, the biologically active features of the pheromone of anxiety were determined depending on the storage term.

RESULTS AND DISCUSSIONS

In order to obtain the pheromone of anxiety from the tissues of worm of California Red Hybrid,a device with two platinum electrodes, a glass of plastic and a power supply was fitted. In the process of controlling the working regimes of the mounted device, it was found that the most efficient one is the one in which the current drop is of 4.5 amps; duration of electrical stimulation - 30 seconds multiplicity - 3 times, stimulation interval - 30 seconds.The maximum amount of pheromone of anxiety, obtained from 50 mature worms, in the result of the triple electrical stimulation constituted 5.0 ml to 6.0 ml. Thus, the average amount of the pheromone of anxiety obtained from a mature worm in the above-mentioned stimulation regime consisted of 0.10 ml - 0.12 ml.

It was found that using ot her working regimes of the device used for electrostimulation, from a worm was obtained a smaller volume of pheromone of anxiety (0.04ml-0.06ml). For

research done for evaluation purposes of the biologically active features were obtained 350 ml of native sterile anxiety pheromone.

Therefore, the volume of the pheromone of anxiety obtained from the tissues of worms the Red Hybrid of California was found in dependence on the electrical stimulation regime.

Subsequently, using the Seitz filter, from the native pheromone of anxiety, the transparent liquid fraction and its solid precipitate were obtained. Both the pheromone samples of native anxiety and its fractions have been subjected to the study of biologically active features towards various microbial cultures.

The results of the investigations carried out on the determination of the bactericidal activity of the native anxiety pheromone and the fractions obtained from it (concentrated and in dilutions) depending on the microbial cultures Staphylococcus aureus, Escherichia coli, Micrococcus luteus, Candida albicans are shown in Table 1.

The analysis of the results of the researches regarding the bactericidal activity of the native pheromone of anxiety, concentrated, towards some microbial cultures found that it showed a higher activity than that of the dilutions used in the experiment. The bactericidal activity depending on Staphylococcus aureus of the dilutions 1:1, 1:5, 1:10, 1:20, 1:50 and 1:100 diminished respectively with 54.71%, 81.88%, 91.30%, 90.76%, 99.09% and 95.83%, in comparison with its value, manifested by the concentrated preparation towards this microbial culture.The highest bacterial activity towards Staphylococcus aureus manifested 1:1 dilutions (250.00%) and 1: 5 (100%), and the lowest dilutions were 1:50 - 5.00% and 1: 100 - 23.00%.

The bactericidal activity of the sample of the concentrated transparent fraction of the pheromone of anxiety depending to Staphylococcus aureus was also higher than in dilutions. Thus, analyzing the obtained results, it was found that the bactericidal activity of the dilutions 1: 1, 1: 5, 1:10, 1:20, 1:50 and 1: 100 obtained from the transparent pheromone fraction of anxiety towards Staphylococcus aureus diminished, respectively by 66.67%, 49.21%, 28.57%, 23.81%, 46.03% and 46.03%.

Table 1. Bactericidal activity of the pheromone of anxiety and its fractions depending on some microbial cultures.

The name of the sample	Bactericidal activity ,%		
	Staphylococcus aureus	Eschechia coli	Candida albicans
The native pheromone of anxiety			
1.Concentrated	552	370	-
2.Dilutions: a) 1:1	250	355	-
b) 1:5	100	70	-
c) 1:10	48	77	-
d) 1:20	51	93	-
e) 1:50	5	51	-
f) 1:100	23	42	-
The transparent fraction of the pheromone of anxiety			
1.Concentrated	63	43	400
2. Dilutions: a) 1:1	42	65	100
b) 1:5	31	49	100
c) 1:10	18	26	100
d) 1:20	15	36	100
e) 1:50	29	20	100
f) 1:100	29	8	-
The pheromone precipitation of anxiety			
1. Initial	986	124	-
2. Kept for a month	77	91	-
3. Kept for two months	69	53	-

Source: Own determinations

The lowest bactericidal activity was 1:20 dilution (only 15%).

The bactericidal activity of the solid precipitate fraction of the pheromone of anxiety was determined, depending on the period of storage in the refrigerator. It was found that the bactericidal activity of samples of the solid precipitate of the pheromone of anxiety depended on the storage period.

Thus, after one month of storage of the precipitate under refrigerator conditions, the bactericidal activity of Staphylococcus aureus decreased by 98.19%, and after two months it decreased by 93 %.

Hence, the precipitate obtained from the pheromone of anxiety must be used

immediately after it is obtained, because then its bactericidal activity towards Staphylococcus aureus is higher.

Bactericidal activity, depending on the microbial culture of Escherichia coli, also depended on the state of the pheromone of anxiety.

Thus, by testing the bactericidal activity of the pheromone of anxiety, native, concentrated and its transparent fraction, depending on the microbial culture of Escherichia coli, demonstrated that it was higher than in dilutions used in the study process.

The bactericidal activity of dilutions of native pheromone of anxiety 1:1, 1: 5, 1:10, 1:20, 1:50 and 1: 100, depending on the Escherichia coli microbial culture, decreased by 4.05% 81.08%, 79.19%, 86.22% and 88.65%, compared to the value of the pheromone of anxiety, native, concentrated.

The bactericidal activity of the concentrated transparent fraction of the anxiety pheromone, depending on the microbial culture of Escherichia coli, was higher than in the dilutions 1:10, 1:20, 1:50 and 1:100. The bactericidal activity of the mentioned dilutions decreased by 39.53%, 16.28%, 53.49% and 81.40%, respectively. The 1:1 and 1:2 dilutions of the transparent fraction of pheromone of the anxiety showed higher bactericidal activity towards the Escherichia coli microbial culture, surpassing that of the transparent concentrated fraction by 51.16% and 13.95%, respectively.

The bactericidal activity of the precipitate towards the microbial culture of Escherichia coli was high in the initial period, and after one month and two months of storage in the refrigerator, it decreased by 26.61% and 57.26%, respectively.

Both the native pheromone of anxiety, concentrated and its dilutions, and the precipitate obtained from it, did not show bactericidal activity towards the Candida albicans microbial culture. Against this culture, showed bactericidal activity, only the transparent fraction of the pheromone of anxiety and its dilutions. The bactericidal activity of all the dilutions (1:1, 1:5, 1:10, 1:20, 1:50, 1:100) of the transparent pheromone of anxiety, depending on the Candida albicans microbial culture, decreased by 75 % as compared to that of its concentrated fraction.

The results of the research on the bactericidal activity of the native pheromone of anxiety and the fractions obtained from it (concentrated and in dilutions) depending on Micrococcus luteus microbial culture are shown in Table 2.

Table 2. Bactericidal activity of the pheromone of anxiety and fractions obtained from it depending on Micrococcus luteus

Name of the sample	Bactericidal activity, %
Pheromone of anxiety native	
1.Concentrated	13,500
2. Diluțions: a) 1:1	3,900
b) 1:5	2,500
c) 1:10	1,500
d) 1:20	400
e) 1:50	200
f) 1:100	100
The transparent fraction of the pheromone of anxiety	
1.Concentrated	400
2. Diluțions: a) 1:1	-
b) 1:5	-
c) 1:10	40
d) 1:20	-
e) 1:50	-
f) 1:100	-
Precipitate of the pheromone of anxiety	
1. Initial	-
2. Kept for a month	-
3. Kept for two months	-

Source: Own determinations

In a result of the study of the bactericidal activity of the various fractions of the pheromone of anxiety towards microbial culture Micrococcus luteus it was found that it manifested it self differently depending on the fractions and dilutions. Analyzing the results outlined in Table 2, it was found that a high bactericidal activity was manifested by the pheromone of anxiety concentrated native, and fractions and dilutions had a lower activity. The bactericidal activity of the dilutions 1: 1, 1: 5, 1:10, 1:20, 1:50, 1:100 obtained from the pheromone of anxiety native towards the microbial culture Micrococcus luteus diminished respectively with 71.11%, 81.48%, 89.89%, 97.04%, 98.52% and 99.29%

compared to the same activity of the native pheromone concentrated.

The bactericidal activity, of the transparent fraction of the pheromone of anxiety depending on Micrococcus luteus microbial culture, diminished by 97.04% compared to that of native pheromone concentrated. Dilutions of this fractions did not show bactericidal activity against Micrococcus luteus, except for the 1:20 dilution, which had a lower activity with 90 % compared to the concentrated transparent fraction.

The pheromone precipitate of anxiety showed no bactericidal activity towards Micrococcus luteus microbial culture.

The lysozyme activity of the undiluted pheromone of anxiety constituted 10.2%, and in the dilutions 1:10 - 1:100 it oscillated from 8.6% to 8.7%, which led to the fact that research would no longer be continued.

CONCLUSIONS

Therefore, analyzing the results of the carried out researches, it was found that the high bactericidal activity was manifested by the pheromone of the anxiety, native concentrated towards the microbial Staphylococcus aureus and Escherichia coli, and towards Candida albicans this was absent.

The transparent fraction of the pheromone of anxiety concentrated manifested high bactericidal activity depending on Staphylococcus aureus, Escherichia coli and Candida albicans, and its dilutions 1:1 and 1: 5 - only towards Escherichia coli.

The obtained precipitate, from the pheromone of anxiety, showed high bactericidal activity only at the initial stage, as kept in refrigerator the bactericidal activity diminished depending on the storage period.

The lysozyme activity of the undiluted pheromone of anxiety and in 1:10 - 1:100 dilutions, depending on microbial culture Micrococcus luteus, manifested weakly, which led to the cessation of researches.

So, from the ones reported, the biologically active particularities of the pheromone of anxiety, depending on some microbial cultures, allow it to be used as an antibacterial preparation in the treatment of certain diseases.

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