THE INFLUENCE OF LOW INTENSITY MILLIMETER WAVES ON THE MULTIPLICATION AND BIOSYNTHETIC ACTIVITY OF *Saccharomyces carlsbergensis CNMN-Y-15* YEAST

Agafia USATÎI*, Elena MOLODOI*, Anatol ROTARU*, Taisia MOLDOVEANU*

* *Institute of Microbiology and Biotechnology of Academy of Sciences of Moldova, Chişinău, Moldova*
Corresponding author: Agafia Usatîi, Institute of Microbiology and Biotechnology, 1, Academiei, 2028 MD, Chişinău, Moldova, phone: 0037322738013, fax: 0037322725754, e-mail: usatyi.agafia@gmail.com; biotehnol_asm@mail.ru

Abstract. The study of the influence of low intensity millimeter waves effects on the multiplication and biosynthetic activity of oleogene components of yeast strain *Saccharomyces carlsbergensis CNMN-Y-15* has been effectuated. It was established that the biological effect of millimeter wave regime depends on the mode, duration of treatment, phase of formation of population, which can serve as one of the factor regulating the multiplication and the biosynthesis of bioactive principles of yeasts.

Keywords: *Saccharomyces carlsbergensis,* millimeter waves, biological effect, multiplication, lipids, sterols

INTRODUCTION

To develop the efficient technologies of obtaining of some scheduled products it is necessary to improve the biosynthetic quality of an producent. There are a number of processes of intensification of the processes of synthesis of bioactive principles from microorganisms [16]. The interaction of biological objects with external electromagnetical field presents a great interest both from the theoretical or practical points of view.

The influence of low intensity millimeter waves on the some biological objects is intensively studied during the last 25 years. Millimeter waves with the wavelength range λ = 1-10 mm correspond to the EHF (extremely high frequency) band, with the frequency of 30-300GHz. This band is placed between the microwave and infrared optical field in the electromagnetic spectrum [18].

It is mentioned that electromagnetical radiation in natural conditions practically does not exist due to the absorption in the atmosphere. Therefore, living organisms have not created mechanisms to adapt to variations in this range during development, the only natural source of millimeter wave as they themselves [21]. So it is important to study the action of millimeter electromagnetic radiation on the biological objects of artificial origin.

Over the past decades, numerous experimental researches were effectuated to evaluate the effect of electromagnetical field on medical-biological objects, as a result the new research direction - millimeter electromagnetobiology have been created.

It demonstrated that the effect of low intensity millimeter waves can occur at different levels of organization of living matter: from cells and sub cellular structures to an organism [1, 7, 10, 12]. In particular, low-intensity millimeter waves have found wide application in medical practice, both diagnostic and the treatment of different diseases [2, 8-9, 17, 32]. It is also proven efficacy in veterinary and agriculture millimeter wave [28].

The positive effect of low intensity millimeter waves has been established for various species of bacteria, microalgae, yeast, actinomycetes, molds and described in numerous scientific publications [6, 29, 31]. It was established that the action of millimeter wave have influenced on the vital activity of microorganisms, cell division, enzyme synthesis, accumulation of biomass, may contribute to morphological changes and alteration of biological properties of organisms, to the mobilization of resource reservation [30]. The changes in morphological, physiological, cultural features, technological indices have been demonstrated as the result of investigations with the yeast *Saccharomyces cerevisiae* strain [26]. The possibility of reducing development cycle and increasing accumulation of biomass during the treatment of *Saccharomyces carlsbergensis* with millimeter waves have been established [20].

So the low-intensity millimeter waves are frequently used in various fields of the economy. Although the mechanisms of millimeter waves generated by modifications on molecular or cellular levels remain partially unclear. There is no single theory that would explain the mechanisms of action of low intensity millimeter wave on the biological systems and no effective methods of evaluation of various methods of adjustment.

The analytical study of literature has allowed to establish the importance of a low intensity millimeter waves in biotechnology of cultivation of micro-organisms. In particular, it is desirable to accumulate new data on field millimeter particular action, both at population and biosynthetic properties.

The aim of this research was to establish the effects of low intensity millimeter waves on the process of multiplication, biosynthesis of lipids and sterols in yeast strain *Saccharomyces carlsbergensis CNMN-Y-15*, results which can serve as a basis for strategy of using millimeter wave low intensity in oleobio-tehnology.

MATERIALS AND METHODS

The yeasts *Saccharomyces carlsbergensis* strain *CNMN-Y-15* (as active producent of sterols) from the Moldavian National Collection of Nonpathogenic Microorganisms were used [14].

Yeast material, grown on a solid medium at 28^0C during 48h was subjected to treatment with millimetre waves. The control tests were in the same conditions without irradiation.

As generator of low-intensity millimeter wave (power flux density 1mW/ cm^2 ÷ 10mW/ cm^2) the device "Явь-1" was used, where the length λ = 5.6 mm (53.8 GHz) under periodically and continuously mode. The duration of treatment has been varied depending on the aim of experience and up to 1, 5, 10, 15, 20, 30, 40, 50, 60 minutes.

Cultivation of yeast was effectuated in Erlenmeyer flasks capacity 1L, containing 0.2 L of the nutritive medium YPG with the following composition (g/l): peptone - 20.0, glucose - 20.0, yeast extract - 10.0, pH =5.5 [5]. Samples were cultivated on rotary shaker (180-200 rpm.), at temperature of 25...27^{0}C for 72h.

The evaluation of low-intensity millimeter waves on the population level was made by photo colorimetrical method [23].

The determination of productivity was effectuated gravimetrically, through the separation of biomass from cultural liquid by centrifugation [25].

Lipid extraction from the biomass and quantitative determination of lipids was performed according to the method Bligh, Dyer and by the new procedure in our modification [4, 13].

Determination of ergosterol was performed according to established procedure [15].

All experiences were performed in three replicates, each variant in three samples. Data were statistically processed by the method of Dospehov, Maximov [24, 27].

RESULTS

The basic biological effects of low intensity millimeter wave actions have been investigated depending on mode, duration of treatment, stage of development of yeast population, double irradiation. Criteria for evaluating of the physiological status of culture irradiated with low-intensity millimeter wave were the following: modifications at the population level, changes in cell membranes (lipid content and ergosterol).

It is known that the stability of monocellular organisms to external factors depends on growth phase and degree of formation of the population. So an important problem in relations of microorganism - millimeter wave presents an equilibrium in the process of formation of the population.

Study of biological effects of millimeter waves on the yeast *Saccharomyces carlsbergensis CNMN-Y-15* strain in two modes - continuously and periodically, has demonstrated that the periodically mode that stimulates the yeast multiplication by 30 - 59.4% was the optimal (Fig. 1). Marked effect was more pronounced in samples irradiated for 1-10 minutes.

An essential factor of the low-intensity millimeter waves action on the yeast population it is the duration of their application. Nine values of duration of irradiation (1 to 60 minutes) with millimeter wave were investigated in continuously and periodically mode. It was found that the maximum effect of stimulating of the multiplication of yeast (with 25.9 to 33.5%) is obtained at millimeter wave treatment for 1-10 minutes, the optimal mode being periodically (Fig. 2).

Another important aspect for yeast cultivation technology is the evaluation of the responsiveness in time of the studied strain to the low-intensity millimeter wave action, issued in different regimes and reveal of the modifications which may arise during the development of the population. It was determined that the continuously action of millimeter waves causes an inhibitory effect on multiplication of culture in the first 48h.

Conditions for periodically mode have an stimulating effect on yeast multiplication (treated with millimeter wave for 10 minutes), strain being more responsive within the first 24 h of cultivation (Fig. 3).

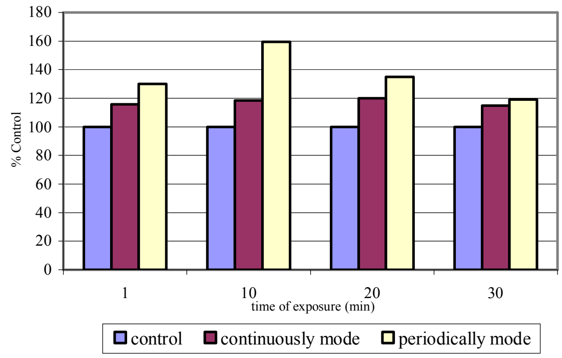

Figure 1. The influence of low intensity millimeter waves on the multiplication of yeast *Saccharomyces carlsbergensis CNMN-Y-15* according to the mode of irradiation.

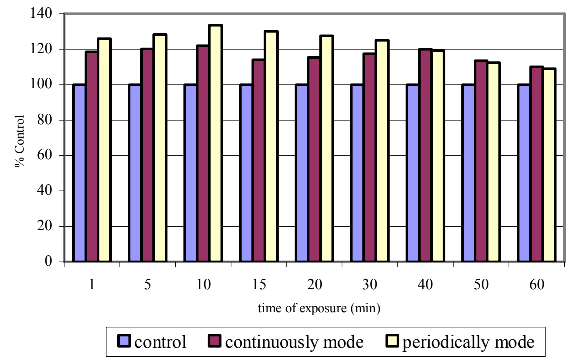

Figure 2. The influence of low intensity millimeter waves on the multiplication of yeast *Saccharomyces carlsbergensis CNMN-Y-15* according to the duration of irradiation.

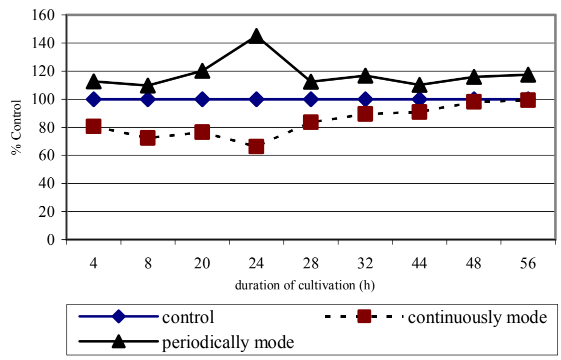

Figure 3. The influence of millimeter waves of low intensity on the multiplication of yeast *Saccharomyces carlsbergensis CNMN-Y-15* according to the continuously and periodically mode.

It was established by the studying of the biological effect of low intensity millimeter waves in the time of 5, 15, 30 minutes that the degree of population stability of *Saccharomyces carlsbergensis CNMN-Y-15* was higher at the irradiation of culture growth in the phase of accelerated growth, cell number remains practically on the blank data (Fig. 4).

Irradiation of the strain in a latent phase stage demonstrates a stable effect of stimulation from 26,8 to 35% of the blank. The phenomenon can be explained by the fact that already formed population have an integral system for maintaining balance in general and so protecting the cell of the action of external factors.

The obtained results are consistent with the literature data about monocellular organisms which are more stable to irradiation in populations already formed, compared with those who are in the early stages of development [22]. Thus, to accelerate the multiplication of yeast culture treatment with millimeter waves of latency phase it is recommend.

Special features of development of *Saccharomyces carlsbergensis CNMN-Y-15* population have been established during the investigations of the effect of treatment of the yeast strain with millimeter waves of low intensity. It was found that repeated irradiation of yeast culture, initial from the latency phase to inoculation and repeatedly accelerated growth phase, causes amplification of the stimulatory effect of cells multiplication (by 30-47%) during the treatment in 5 to 30 minutes (Fig. 5).

The study of sensitivity of various components of the cell membrane to low intensity millimeter waves action presents the both theoretical and practical interes. Some authors indicate ambiguous effect of low intensity millimeter waves on the functional status of the cell, in particular biosynthetic process [6].

The effect of low-intensity millimeter waves on the functional activity of the yeast cell membranes, in particular lipid phase is less studied.

Since the studied strain of yeast has practical importance for biotechnology as an active producer of sterols, the next stage of research was the effect of low intensity millimeter waves on the process of biosynthesis of bioactive principles, in particular, the lipid content and sterols.

The study the influence of mode of low-intensity millimeter waves treatment have demonstrated that continuously mode does not influence significantly on lipid biosynthesis in the biomass of yeast *Saccharomyces carlsbergensis CNMN-Y-15*.

A stimulation of lipid biosynthetic process, and particularly sterols fraction have been established at irradiation under periodically mode (20 minutes), the contents of which exceeds 53% and 75% the blank (Fig. 6). These results indicate the opportunity of using of the periodically mode to achieve biological effects, particularly on the process of biosynthesis of lipids.

It is indicated that the effects of low intensity millimeter waves on biosynthetic processes are depending on the duration their influence on biological object [31]. The obtained results confirmed the dependence of biological effect on the duration of treatment of the studied yeast strain with low intensity

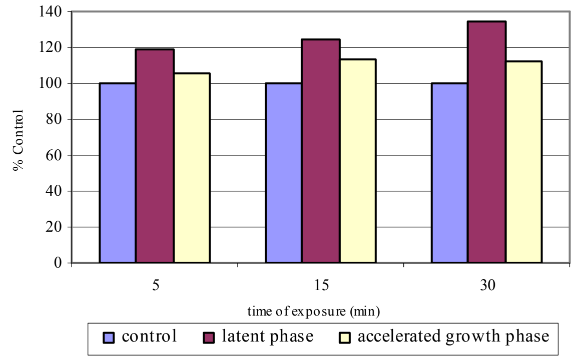

Figure 4. The influence of millimeter waves of low intensity on the multiplication of yeast *Saccharomyces carlsbergensis CNMN-Y-15* according to the phase of growth.

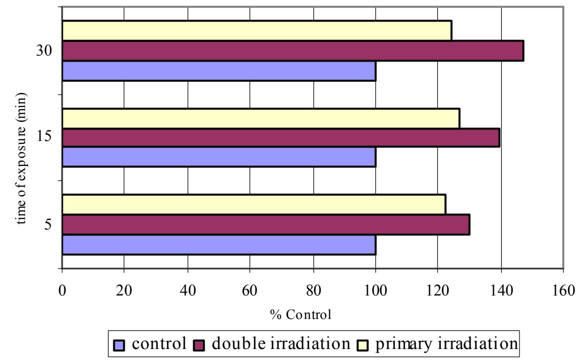

Figure 5. The influence of duble treatment of millimeter waves of low intensity on the multiplication of yeast *Saccharomyces carlsbergensis CNMN-Y-15.*

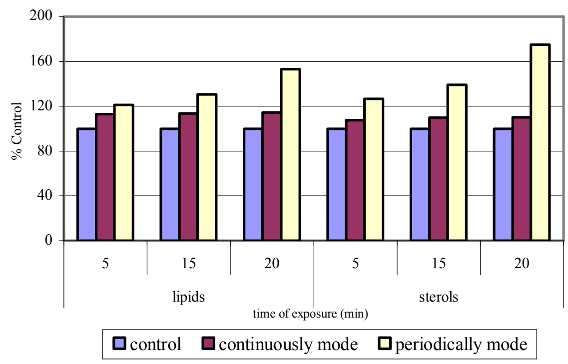

Figure 6. The influence of millimeter waves of low intensity on the lipids and sterols biosynthesis in biomass of yeast *Saccharomyces carlsbergensis CNMN-Y-15* according to the mode of treatment.

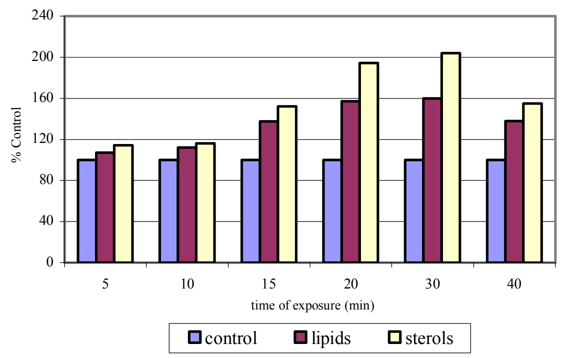

Figure 7. The influence of millimeter waves of low intensity on the lipids and sterols biosynthesis in biomass of yeast *Saccharomyces carlsbergensis CNMN-Y-15* according to the duration of treatment.

millimeter waves. It was established the optimal duration of treatment of strain 20-30 minutes (57-60% for lipids and 94-104% for sterols) (Fig. 7).

DISCUSSION

Mechanisms of millimeter wave interaction with living organisms are still unclear. Contradictory date of the athermal action of millimeter waves on biological objects allow us to display only certain assumptions. It submitted a view that the specific features of millimeter radiation action on living organisms is effectuated due to their role of information signals, which regulates vital activity of organism in the resonant conditions [18]. It appears that low intensity millimeter radiation is a universal mechanism for transmitting information between the living organisms. This assertion is based on two fundamental principles: the ability of living organisms to generate internal irradiation and to react to external irradiation.

The establishing of the structural units that react directly to external factors present one of the main objectives of the studying the mechanisms of field interaction of electromagnetical field with biological objects. According to the authors, biomembranes presents that element of reception of millimeter waves [33].

Betski and co-authors consider that the first target for millimeter waves is water-soluble matrix of organism through which excitation is transmitted to the molecular level as an information signal [3, 11, 19].

So, the theoretical significance of the obtained results consist of the fact that low-intensity millimeter waves can be used as one of regulating factor of the principles of multiplication and biosynthesis of the *Saccharomyces carlsbergensis CNMN-Y-15* and solve the problems related to the elaboration of technologies of obtaining of the bioactive components of the studied strain of yeast.

The biological effect of low intensity millimeter wave action on the yeast *Saccharomyces carlsbergensis CNMN-Y-15* investigated at the population and cellular level may cause stimulation or inhibition of microorganisms activity or may remain neutral, fact that depends on the regime and duration of treatment, phase of population formation, and number of treatments with millimeter waves. The obtained results were used for the elaboration of processes of increasing of the productivity of the yeast *Saccharomyces carlsbergensis CNMN-Y-15* and contents of bioactive substances in the biomass with the utilization of low intensity millimeter waves as a stimulating factor.

REFERENCES

[1] Belyaev, I., Shcheglov, V., Alipov, E., Ushakov, V., (2000): Nonthermal effects of extremely high-frequency microwaves on chromatin conformation in cells in vitro-dependence on physical, physiological, and genetic factors. IEEE Transactions on Microwave Theory and Techniques, 48(11): 2172-2179.

[2] Beneduci, A., (2009): Evaluation of the Potential In Vitro Antiproliferative Effects of Millimeter Waves at Some Therapeutic Frequencies on RPMI 7932 Human Skin Malignant Melanoma Cells. Cell Biochemistry and Biophysics, 55(1): 25-32.

[3] Betskii, O., Ghitsu, D., Rotaru, A., Ciobanu, N., (2003): The non-linear dielectric permitivity during excitation of coherent excitation coherent phonons in biological objects. 13th Russian Symposium Millimeter Waves in medicine and biology, Moscow, pp. 118-121.

[4] Bligh, E.G., Dyer, W.J., (1959): A rapid method of total lipid extraction and purification. Biochemistry and Cell Biology, 37(8): 911-917.

[5] Gao, H., Tan, T., (2003): Fed-batch fermentation for ergosterol production. Process Biochemistry, 39: 345-350.

[6] Garkusha, O., Mazurenko, R., Makhno, S., Gorbik, P., (2008): Influence of low-intensity electromagnetic millimeter radiation on the vital activity of *Saccharomyces cerevisiae* cells. Biophysics, 53(5): 402-405.

[7] Kalantaryan, V., Babayan, Y., Gevorgyan, E., Hakobyan, S., Antonyan, A., Vardevanyan P., (2010): Influence of low intensity coherent electromagnetic millimeter radiation (EMR) on aqua solution of DNA. Progress in Electromagnetics Research Letters, 13: 1-9.

[8] Logani, M., Agelan, A., Ziskin, M., (2002): Effect of millimeter wave radiation on catalase activity. Electromagnetic Biology and Medicine, 21(3): 303-308.

[9] Logani, M., Szabo, I., Makar, V., Bhanushali, A., Alekseev, S., Ziskin, M., (2006): Effect of millimeter wave irradiation on tumor metastasis. Bioelectromagnetics, 27(4): 258-264.

[10] Reshetnyak, S., Shcheglov, V., Blagodatskikh, V., Gariaev, P., Maslov M., (1996): Mechanisms of interaction of electromagnetic radiation with a biosystem. Laser Physics, 6(4): 621-653.

[11] Rotaru, A., (2002): Coherent electromagnetic field interaction with biological media. 12th National Conference of the Romanian Physical Sosiety Trends in Physics, Târgu-Mureş, pp. 94.

[12] Szabo, I., Rojavin, M., Rogers, T., Ziskin, M., (2001): Reactions of keratinocytes to in vitro millimeter wave exposure. Bioelectromagnetics, 22: 358-364.

[13] Usatîi, A., Calcatiniuc, A., Grosu, L., Şirşov, T., (2002): Procedeu de extragere a lipidelor din drojdii. MD 1930, BOPI nr. 5/2002, pp. 26-27.

[14] Usatîi, A., Molodoi, E., Moldoveanu, T., Borisova, T., Topală, L., (2008): Tulpină de drojdie *Saccharomyces carlsbergensis* – sursă de steroli. MD 3538, BOPI nr. 3/2008, pp. 32-33.

[15] Usatîi, A., Chiriţa, E., Molodoi, E., Moldoveanu, T., Cucu, T., Borisova, T., (2008): Procedeu de obţinere a ergosterolului din drojdii *Saccharomyces*. MD 3570, BOPI nr. 4/2008, pp. 41-42.

[16] Usatîi, A., Molodoi, E., Moldoveanu, T., Borisov, T., Chiseliţa, N., (2009): Elaborarea mediilor de cultură pentru *Saccharomyces carlsbergensis CNMN-Y-15* producător de steroli. National Conference: Probleme actuale ale microbiologiei şi biotehnologiei, 5-6 Oct 2009, Chişinău, Moldova, pp. 179-181.

[17] Usichenko, T., Edinger, H., Gizhko, V., Lehman, C., Wendt, M., Feyerherd F., (2006): Low-Intensity Electromagnetic Millimeter Waves for Pain Therapy. Advance Access Publication, 3(2): 201-207.

[18] Бецкий, О., Девятков, Н., (1996): Электромагнитные миллиметровые волны и живые организмы. Биомедицинская радиоэлектроника, 3: 4-10.

[19] Бецкий, О., Лебедева, Н., Котровская, Т., (2002): Стохастический резонанс и проблема воздействия слабых сигналов на биологические системы.

Миллиметровые волны в биологии и медицине, 3(27): 3-11.
[20] Бецкий, О., Кислов, В., Лебедева, Н., (2004): Миллиметровые волны и живые системы. Сайнс Пресс, Москва, 235 p.
[21] Бецкий, О., Лебедева, Н., (2004): Синергетика и электромагнитные поля. Миллиметровые волны в биологии и медицине, 4(36): 5-16.
[22] Гапочка, Л., Гапочка, М., Королев, А., (2002): Популяционные аспекты устойчивости одноклеточных организмов к действию лектромагнитного облучения низкой интенсивности. Миллиметровые волны в биологии и медицине, 2(26): 3-9.
[23] Герхард, Ф., (1983): Методы общей бактерологии. Мир, Москва, 536 p.
[24] Доспехов, Б., (1985): Методика полевого опыта. Агропромиздат, Москва, 351 p.
[25] Егоров, Н., (1995): Руководство к практическим занятиям по микробиологии. МГУ, Москва, 224 p.
[26] Крыницкая, А., Суханов, П., Седельников, Ю., Астраханцева, М., Гамаюрова, В., (2004): Влияние последействия КВЧ-излучения на активность хлебопекарных дрожжей. Миллиметровые волны в биологии и медицине, 4(36): 17-27.
[27] Максимов, В., (1980): Многофакторный эксперимент в биологии. МГУ, Москва, 280 p.
[28] Петров, И., Морозова, Э., Моисеева, Т., (1991): Стимуляция процессов жизнедеятельности в растениях микроволновым облучением. В сбор. докладов межд. симп. Миллиметровые волны нетепловой интенсивности в медицине и биологии., Москва, 2: 502-504.
[29] Реброва, Т., (1992): Влияние электромагнитного излучения миллиметрового диапазона на жизнедеятельность микроорганизмов. иллиметровые волны в биологии и медицине, 1: 37-47.
[30] Тамбиев, А., Кирикова, Н., (2000): Некоторые новые представления о причинах формирования стимулирующих эффектов КВЧ-излучения. Биомедицинская радиоэлектроника, 1: 23-33.
[31] Тамбиев, А., Кирикова, Н., Лукьянов, А. (2002): Применение активных частот электромагнитного излучения миллиметрового и сантиметрового диапазона в микробиологии. Наукоемкие технологии, 1: 34-53.
[32] Шмелева, Т., Послaвский, М., и др. (1991): Повышение фагоцитарной активности лейкоцитов крови больных язвенной болезнью желудка после облучения миллиметровыми волнами. Миллиметровые волны в медицине. Сбор. ст. Под ред. акад. Н.Д. Девяткова и проф. О.В. Бецкого. Москва, 1: 240-245.
[33] Яшин, А., (2000): Явление стохастического резонанса в биосистемах при воздействии внешнего электромагнитного поля и его роль в регуляции свободной энергии. Physics of the Alive, 8(2): 14-28.

Received: 10 June 2010
Accepted: 3 September 2010
Analele Universității din Oradea – Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433