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Millimeter waves in the newest agricultural biotechnologies

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Abstract

The present article is devoted to the analysis of being available experimental and theoretical data on effects of millimeter electromagnetic radiation on seeds, animal sperm, microalgae. In the article the effectiveness of the action of millimeter radiation on the seed germination and seedling growth was examined, on increase in mobility spermatozoa and on duration of their existence, on increase in a target biomass of microalgae and pigments.

1 Introduction

In contrast to chemical methods, millimeter waves (MMW) radiation is environmentally appropriate technology what is wery important for farming industry. In addition, the possibility to increase the productivity (crop yield, milk yield and biomass yield) is always topical in agriculture.

Favorable results were obtained in the application of millimeter waves in biotechnology. The first works in this direction have demonstrated that

MMW irradiation changes the character of vital functions in microorganisms: it affects the cell division, enzyme synthesis, growth rate and yield of biomass, and may cause morphological changes and changes in the biological properties of microorganisms. Thus MMW can change the parameters of vital functions in microorganisms, regulare their physiological activity, and mobilize their resources [1–8].

2 The review of theoretical researches

During irradiation of biological objects containing water which is present partially in the free state and partially in composition with organelles in corresponding biosystems, it is generally agreed that 50% of the absorbed dose in the "middle cell" goes for water, while the other 50% are accounted for its organelles and dissolved substances. In accordance with localization of consumed energy (in water or main substance) it is possible to discuss direct and indirect action of electromagnetic radiation [9–11].

During interaction of electromagnetic radiation with water knocking out of electrons from water molecules finally occurs forming so called molecular ions having positive and negative charges. Schematically this process can be represented in the following way:

$$\begin{aligned} H_2O &\rightarrow H_2O^{+}+e_1, \\ H_2O^{+}+e_1 &\rightarrow H_2O^{-}. \end{aligned}$$

Appearing water ions in their turn decompose forming a number of radicals which interact with each other:

$$\begin{aligned} H_2O^{+} &\rightarrow H^{+}+OH, \\ H_2O^{-} &\rightarrow H^{-}+OH, \\ H+OH &\rightarrow H_2O, \\ OH+OH &\rightarrow H_2O_2, \\ H_2O_2+OH &\rightarrow H_2O+HO_2. \end{aligned}$$

It is considered that the main effect of electromagnetic action is determined by such radicals as H, OH and mainly HO_2 (hydroperoxide). The last radical possessing high oxidation ability is formed during water production in the presence of oxygen: $H + O_2 = HO_2$. In this case the following reactions occur:

$$
\begin{aligned}
OH+OH &\rightarrow H_2O_2,\\
H_2O_2+OH &\rightarrow H_2O+HO_2,\\
H_2O_2+O_2H &\rightarrow H_2O+OH+O_2,\\
H+O_2 &\rightarrow HO_2,\\
HO_2+OH &\rightarrow H_2O+O_2.
\end{aligned}
$$

Radicals appearing as a result of radiation interaction with water interact with dissolved molecules of different compounds giving rise to radical products for the second time. Life-span of these products is considerably longer when compared to life-span of primary radicals, so they are capable of manifesting higher selectivity of action. Ways of energy changes under the action of ionizing radiations can be shown at the first stage by appearance of ionized and excited molecules:

$$A \rightarrow A^{+}+e_{-1};\ A \rightarrow A^{*}.$$

The most important processes leading to chemical transformations (and, consequently, to attacks) of biomolecules are the following ones:

- dissociation A^* for corresponding molecular products;
- interaction with sensibilizer (e.g. O_2).

It is important to note that as a result of direct and indirect electromagnetic radiation action on biosubstrate *identical* secondary radicals appear which can serve as explanation of a definite specificity of chemical transformations in cell metabolism.

Formation of peroxides occurs during electromagnetic radiation action on lipids, this process being considered a very important one in cell metabolism development. Reaction scheme in this case can be presented as follows:

initial radical formation

ROOH-R*
ROOH-ROO*

chain reactions

R^*+0_2-$R0^*_2$
R00*+RH-R00H+R*

Together with some stimulation of lipid synthesis, increase in lipid's oxidation ability occurs leading to peroxides production. In this case peroxide production is determined not only by the direct radiation action, but as the result of suppressing a number of anti-oxidants. It is necessary to note that results of radiation are revealed as changes in intracellular structures of lipoproteins, in mitochondria and microsomes in particular.

Above-mentioned changes in molecular structures occurring as a result of irradiation are far from embracing all the information existing at present in this field. At the same time, the information available up to the present time is still of descriptive, qualitative nature.

Peroxide oxidation of lipids (POL) is known to represent a chain, free-radical oxidation process including non-saturated fatty acids which constitute a part of phospholipid molecules in cells and playing a leading role during radiation damages, during intoxications and other pathological states of the organism. Permeability of native membranes for ions and other molecules increases as a result of phospholipid's oxidation.

Thus, the majority of existing studies points to cellular membranes of different objects (from plant membranes to erythrocyte ones) as the main location of mm range radiation application where primary mechanisms develop which determine the end effects. According to different authors only the essence of mechanisms occurring in membranes is different.

According to our opinion, the fact that the influence of electromagnetic radiation in mm range is absent in the presence of O_2 points to storage of membranes and free radical states in the lipid phase, thus making this stage highly important for the action of radiation in mm range when compared with convective matter transfer.

Multiple experiments have shown that with prokaryotic and eukaryotic photosynthetic organisms small quantity of energy absorbed at one time causes such important effects as increase in growth, increase in biomass output for 2-2.5 times, increase of pigment quantity in cell for 3.5 times as well as the increase of the organic compounds excretion level into the medium, all this, according to our opinion, certifies the presence of self-accelerating mechanisms for advancing the consequences of radiation.

Experimental data have justified the point of view that primary effect of radiation in mm range consists in changing the membrane permeability and its transport properties. Presence of oxygen seems to be important for initiating changes in functional state of membranes, their permeability and for developing reactions retarded in time which occur according to the principle of self-acceleration, - all of them being main causes of pronounced stimulating action of radiation in mm range on photosynthesizing objects.

Similar mechanisms have been described earlier, e.g., with development of primary reaction of radiation damage under the action of γ-radiation and represented reactions of chain autocatalytic type. A number of facts support this idea, in particular the non-commensurability of the quantity of the energy absorbed under the action of γ-radiation and the organism response, which even in the 1950s permitted to present a hypothesis about the decisive role of lipid chain oxidation in ionizing radiation influence and then on the influence of other damaging factors.

A large school of chemists has shown that the process of lipid peroxide formation with such phenomena possesses a chain, free-radical mechanism, generally characteristic for organic compounds oxidation by molecular oxygen itself.

This doesn't mean an equality in the functioning mechanisms of radiation in mm range and ionizing radiation, but some external manifestations are identical. Ultraviolet radiation seems to possess the same mechanism causing free radicals formation in biological systems and storage of peroxides. Number of authors note that ultraviolet radiation leads to changes in ion transfer through the membrane; peroxide oxidation of lipids in membranes leads to increase in their permeability with comparatively low doses of radiation. Peroxide oxidation is known to be connected with oxygen consumption, that's why when studying the influence of ultraviolet radiation on biomolecular phospholipid membranes, radiation in argon atmosphere appeared to be less effective than that in the presence of dissolved oxygen.

Oxygen presence during radiation in mm range is possibly responsible for further formation and storage of radical and peroxide states and for development of autocatalytic reactions of chain type occurring in the cell lipid phase, mainly in membranes with storing the end products leading to changes in their functional state similar to what appeared earlier with ionizing radiation influence.

Thus, the effect of hydrogen peroxide formed which is a strong acceptor of electrons able to regulate functioning of many fermentative systems can be considered as one of the causes of non-thermal influence of radiation in mm range on biological objects. Presence of peroxides formed could have intensified photosynthesis processes together with pigments storage what we have observed as a result of radiation in mm range.

The above said coincides with the point of view that photosynthetic oxygen is formed not out of water, but of hydrogen peroxide having exogenous or endogenous origin, hence increase of peroxides in the cell under the action of some factors could have corresponded to photosynthesis intensification.

Some authors believe that biological membrane is the most important point of this radiation application because low intensity of microwave radiation leads to acceleration or suppression in active ions transport, to changes in biological membranes permeability due to proteins irradiation in the cell membrane and protein component in the complex of ATP synthesis.

The majority of researchers have shown that cell membranes of different objects (from plant cells to erythrocytes) serve as the main location of influence for radiation in mm range: primary mechanisms which determine final effect of radiation in mm range influence are developed in the membranes.

Thus, electromagnetic radiation in mm range and of low intensity is capable of causing the biosystems reaction which is impossible in case of electromagnetic radiation of high intensity.

Experiments conducted in Israel, Russia, Ukraine have shown that formation of additional radical and peroxide groups leads to stimulation of cell metabolism including increase of mitochondrial membranes potential, acceleration in ATP synthesis, thus leading to the increase of microorganisms biomass, halobacteria *Halobacterium salinarium* among them. Quantity of bacteriorhodopsin obtained depends on the conditions of halobacteria cultivation. Under conditions of oxygen deficiency in halobacteria purple membranes synthesis of protein bacteriorhodopsin occurs which permits halobacteria breathing due to photophosphorylation . Experiments by different scientists have proved the intensity of bacteriorhodopsin formation to depend on the value of media illumination, period of non-radioactive electromagnetic radiation of low intensity and oxygen quantity in the medium [12–15].

Biological effect of applying non-radioactive electromagnetic radiation of mm range and low intensity to photosynthesizing microorganisms is manifested in increase of their biomass which is obtained during cultivation, as well as in increase of pigment content in their cells.

3 Experimental studies in processes of electromagnetic irradiation of biological objects for agriculture

Statistically stable experimental data of numerous experiments on irradiating different biological objects by electromagnetic waves of millimeter (mm) range allows us to state the advantages of applying electromagnetic radiation of mm range to biological objects as an environmentally safe biotechnology for stimulating their biomass increase and growth acceleration. This method appears to be the most perspective one for photobiotechnology. Equipment of mm range developed possesses the following advantages: low inherent power consumption, low weight and small size, simplicity and reliability of maintenance, availability for multi-purpose application, quick recoupment of expenditures for purchasing the equipment.

3.1 Technology and equipment for electromagnetic stimulation and disinfection of plant seeds

At present the ability of simultaneously generating the effects of biostimulation, disinfection and disinsectization during irradiation of seeds from different agricultural plants by electromagnetic waves of mm range has been discovered and experimentally proved. Latest achievements in this field permit to develop new wave technologies for pre-sowing seed treatment and agricultural produce disinfection as well as to manufacture new equipment for practical application of these wave technologies.

The tested effects of these wave technology applications consist in increasing the harvest biomass with preservation of produce quality, decrease in phenophase period and produce disinfection.

During 1992-2006 field and laboratory studies have been carried out with the aim of determining working regimes of microwave action on main culture seeds in the regions of Ukraine, Moldova, Russia. In this case data on manifesting the effects of seeds disinfection under conditions of processing and storage as well as direct results of the influence of electromagnetic field (EMF) of mm range waves (MMW) on biochemical properties of produce have been obtained. The results obtained serve as a basis for development and creation of new MMW equipment with the purpose of treating seeds under steady and field conditions.

Seeds treating by MMW is proposed as one of the methods for increasing quality characteristics of agricultural plants and, finally, for increasing harvesting. Economic feasibility and simplicity are the advantages of irradiation by MMW. Thus, for treating 1 ton of grain about 200 KJ of electric energy are consumed. But the main advantage of irradiation by MMW consists in the ability of improving growth factor and development due to mobilization of inner reserves in the seeds themselves, without chemical treatment or application of genetic engineering methods.

Regimes of treatment which permit to attain maximum effect with a number of grain cultures have been determined experimentally. At present, the following response reactions of seeds to MMW irradiation can be noticed:

1. With correct choice of parameters for seed treatment (oscillation frequency of electromagnetic field, time, power) qualitative characteristics of plants developing out of such seeds are improved.

2. MMW can increase the viability of planting material.

3. Reaction of seeds from different cultures and sorts is different.

4. Field comparative studies have shown the possibility of shortening the phenophase (period of ripening) and increase in harvest quantity (increase in green mass) with treated seeds in relation to control ones.

It has been found by means of biochemical analyses that after treating seeds in the regime of biostimulation they show noticeable stimulation in protein synthesis and increase in acid phosphotase. At the same time it has been found that exceeding the optimum parameters of treatment leads to seed growth retardation.

Attempts in explaining the phenomena occurring in biological objects under the action of MMW of EMF have been taken long ago. However, due to functional complexity of living cells, inability of direct control of physical-chemical factors of metabolic activity in biological tissue, as well as due to difficulty in carrying out precise measurements of MMW of EMF parameters and local values of physical parameters of the material treated (such as temperature, pressure, moisture content), unique answers have not been obtained up to now.

Main results of MMW action on plant tissue consist in the following:

- laboratory germinability and energy of germination is considerably increased when treating non-standard grain;

- effective germination of seeds after their seeding into soil in all cases increases the same data for control specimens (in absolute data from 2.4 to 12.5% depending on the kind and initial quality of seeds);

- periods of phenophases for plants are shortened.

Effects of MMW influence on biological tissue of plant cell have been investigated aimed at determining the causes of growth intensification and hardiness of plants obtained from treated seeds. The following facts served as the basis for determining the mechanism of MMW influence on biological objects: electromagnetic field can act only on electrically charged particles or polar molecules; in biological tissues only water molecules possess high polarity of charges; conversion of MMW electromagnetic energy occurs mainly due to the process of polar molecules interaction; changes occurring in grain do not interfere with prolonged conservative storage. Analysis of the available data led to a supposition that mechanical "cleaning" of plant tissue capillary system (intercellular structures, plasmatic membrane pores, etc.) is the most probable cause of biostimulation. The experiments with wheat seeds of soft and hard kinds in a number of regimes justify this hypothesis. However, prolongation of exposure eventually led to dying out which is most probably connected with phenomena caused by the increase in energy action.

Complex research on studying the MMW influence on isolated strains of phytopathogenes (7 kinds) has been conducted for the first time. Treatment regimes leading to phytopathogenes inhibition or their extinction have been determined by varying the dose of MMW action.

Direct experiments have shown the effectiveness of MMW action on phytopathogenes, regimes of seeds biostimulation and phytopathogenes inhibition coinciding in many variants (combinations of seed and phytopathogene kinds).

At present chemical treatment of seeds and soil is carried out for increasing seed germination and soil protection from pests. A device for pre-sowing seeds treatment is proposed for radically changing the soil and air ecological condition, where charged seeds are irradiated by electromagnetic fields of different frequencies and of low intensity during a short period of time.

The experiments conducted have shown that, after sowing, irradiated seeds germinate for 15-20% quicker and that during ripening the plants grown out of irradiated seeds are for 10-15% less subjected to diseases or die due to pests, thus giving harvest increment (e.g. for wheat the increase is up to 14,420-16,200 kg per ha).

The device can be used for seeds treatment at the initial stage of storage and during storage.

The given device differs from the existing devices of seeds preparation for sowing by application of non-radioactive millimeter electromagnetic radiation of low intensity, by its low cost, by increase in harvest biomass with preservation of produce (grains) quality, by shortening phenophase period, by pests-insects elimination, by disinfecting produce (grains), by environmental safety of irradiated seeds and growing plants, as well as by saving power expenditures for 30-40% during seeds treatment and by saving water discharge for 23-35% during plant watering, by lower self-cost of the harvest obtained.

As an example, let us take experimental data of treating wheat seeds by non-radioactive electromagnetic radiation of mm range (40-70 GHz) and low intensity (2-5 microWatt per sq cm):

	With treatment	Without treatment
Plant height, cm	79.9	74.5
Length of the main ear, cm	9.1	8.9
Number of grains in an ear, pieces	47.0	45.6
Grain weight in an ear, g	1.74	1.44
Weight of 100 grains, g	37.0	31.6
Biological harvest, t/ha	9.47	7.83

Laboratory and semi-industrial tests of an attachment model have been carried out in Russia, the Ukraine and Israel. The device (installation) consists of standard microelectronics elements and standard electromechanical and hydraulic units. The design of an industrial installation includes:

1. Belt conveyor which delivers grain to a loading bunker of a sowing machine (grain drill) and on the surface of which irradiation of seeds takes place,

2. A source of non-radioactive electromagnetic radiation,

3. A power block,

4. A rack for radio-electronic equipment which is situated over the middle part of the belt conveyor.

Seeds irradiation occurs when electromagnetic energy of the source is radiated through a horn aerial directly during operation of a conveyor loading the bunker of a sowing machine (grain drill). Irradiation regimes are determined by geometrical sizes of the seeds, conveyor speed and intensity of the source.

The device is simple and easy to use. One-two operators are needed for maintenance. Depending on the quantity of seeds to be irradiated and plant kind, approximate cost of a home device for pre-sowing seeds preparation in small volumes is equal to $500-700, and that of an industrial device for pre-sowing seeds treatment in big volumes is $ 6000–9000. Recoupment period for a home device is 1.4-1.9 years, and that of an industrial device is 0.8-1.2 years [16–19].

3.2 Technology and equipment for electromagnetic stimulation of animal and bird spermatozoids activity

At present effectiveness of artificial insemination of animals and birds is rather low. Due to this, artificial insemination is repeatedly carried out for 2-3 times, thus increasing the time for obtaining the new offspring and making the output produce more costly. To increase spermatozoid activity with artificial insemination a device for stimulating spermatozoid activity has been developed by means of which irradiation of spermatozoids by non-radioactive millimeter electromagnetic radiation (50–80 GHz) of low intensity (1–3 microWatt per sq cm) at specific frequencies is carried out during a definite period of time.

There exists a model of the device which can be used for tests. The device itself consists of standard mechanical and electronic elements. The experiments conducted in Israel and the Ukraine have shown that, with spermatozoids irradiation by non-radioactive electromagnetic radiation of mm rang, activity of spermatozoids increases for 1.8-2.1 times, spermatozoids life-span increases for 1.5-1.7 times. This increases the effectiveness of artificial insemination from 18% to 63%.

The given development differs from the existing devices for spermatozoid activity stimulation by application of non-radioactive electromagnetic radiation of low intensity, by its low cost, by environmental safety of spermatozoids irradiation process as well as by saving power expenditures and by lowering self-cost of artificial insemination.

Laboratory and semi-industrial tests of an attachment model have been carried out in Russia, Ukraine and Israel. The device (installation) consists of standard microelectronic elements and standard electromechanical and hydraulic units. The design of an industrial installation includes:

1. Post of sperm freezing (defrosting),
2. A source of non-radioactive electromagnetic radiation,
3. A power block,
4. A rack for radio-electronic equipment.

Sperm irradiation occurs when electromagnetic energy of the source is radiated through a horn aerial directly during operations with the sperm (on freezing or defrosting). Irradiation regimes are determined by animal species, sperm volume and intensity of the source.

The device is simple and easy to use. One-two operators are needed for maintenance. Depending on the sperm doses to be irradiated and animal species, approximate cost of a laboratory (home) device for treating small number of doses is equal to $3500–4700, and that of an industrial device for treating a large number of doses is $16000–19000. Recoupment period for a home device is 1.4-1.9 years, and that of an industrial device is 0.8-1.2 years [20].

3.3 Technology and equipment for electromagnetic stimulation of microalgae growth

Our studies have for the first time shown the perspectiveness of using short wavelength radiation for stimulating microalgae and cyanobacteria (pro- and eukaryotes) growth, including such photobiotechnological objects as *Spirulina platensis* and *Platymonas viridis*. To attain this we have first developed the technique of continuous irradiation of cyanobacteria and microalgae and have carried out the optimization of irradiation parameters. We have obtained a statistically reliable stimulating effect with single irradiation characterized by time, frequency and power dependence which was accompanied by intensive biomass stockpiling. Interaction of short wavelength radiation with cyanobacteria and microalgae had a pronounced resonance character.

Mechanisms of primary interaction between short wavelength radiation and biological objects have not been discovered yet, but on the basis of our data and literature it can be supposed that changes in the condition of cellular membranes and their lipid phase in the first place, lie in the basis of influences stimulating cyanobacteria and microalgae growth.

As experiments conducted in Russia and Israel have shown, short wavelength radiation can be used as a new environmentally safe physiological regulation of cell metabolism with photosynthesizing organisms.

The proposed development represents an attachment to bioreactors and fermenters where photosynthesizing biological objects (microalgae, yeasts, bacteria, viruses) are cultivated.

In order to increase microalgae and producers biomass output as well as shorten the time of biotechnological process, a device has been developed which, according to a preliminary introduced program, carries out irradiation at definite moments in the cycle of microalgae cultivation. In this case parameters of electromagnetic radiation of low intensity are automatically changed depending on the time of cultivation.

The experiments conducted in Israel and Russia have shown that microalgae biomass steadily increases for 250-400% and producers biomass increases for 180-300%. In this case oxidizing ability (activity) of irradiated microalgae cultural medium ranges up to 264%, while that of non-irradiated microalgae attains only 110%.

The given development differs from the existing devices for stimulating increase in cultivated biological object biomass by application of non-radioactive millimeter electromagnetic radiation (40–120 GHz) of low intensity (3–8 microWatt per sq cm), by increase in bioreactor (fermenter) productivity for 2-4 times, by better quality of output produce, by environmental safety of output produce as well as by saving power and water for 30-40% and by lower self-cost of output products in biotechnological process..

Laboratory and semi-industrial tests of an attachment model have been carried out in Russia, Ukraine and Israel. Increase in microalgae biomass is observed in phases cultivation (D) and stress (S) during the industrial process of microalgae cultivation. As follows from data of experiments weight of microalgae biomass *Haematococcus pluvialis* which has been irradiated before phases D and S attained correspondingly 119% and 141% of microalgae biomass control group. Data of experiments show that the weight of isolated (produced) pigment Astaxanthin was equal to 187% (on the 7th day of cultivation) and 219% (on the 9th day of cultivation) of the weight of pigment isolated from microalgae control group.

Data of experiments show that in case the physiological solution was irradiated before phase D and then added to culture medium with microalgae, biomass of such microalgae group increases with further cultivation (in phase D) for 150-187% in comparison to the control biomass of microalgae group which wasn't subjected to radiation. Biomass of such cultivated microalgae group increases for 1.55 times in comparison to microalgae group which was completely irradiated before phase D.

Absence of non-specific initial conditions influence on cultivation process shows stable manifestation of biological effects with millimeter irradiation which was certified by experimental data obtained during an experiment using the same equipment of industrial tubular bioreactor in August and September 2003 (at different meteorological conditions).

Low intensity of radiation (3–8 microWatt per sq cm) and short duration of radiation (short time of radiation exposure) (up to 60 minutes) makes it possible to state that vigorous growth of microalgae and pigment biomass is caused not by temperature increase but only by the influence of millimeter electromagnetic radiation on cultivated medium.

The results of semi-industrial experiments conducted in Israel completely certified biological effects of waves in mm range which have been obtained in Russia and Ukraine during laboratory tests. These tests and semi-industrial experiments carried out for the first time, have shown that irradiation by MMW causes intensive development in cultures of photosynthesizing microalgae and their pigments.

The device (installation) consists of standard microelectronic elements and standard electromechanical and hydraulic units. The device is simple and easy to use. One operator is needed for maintenance. Depending on the volume of cultivated medium, kind of algae, approximate cost of a small volume bioreactor attachment is equal to $ 5000–7000, and that a large volume bioreactor is $ 130,000–180,000. Recoupment period for a small volume bioreactor attachment is 1.4-1.9 years, and that for the owner of a large volume bioreactor is 0.8-1.2 years [21–23].

4 Conclusion

Experimental results of studying the influence of millimeter waves on biological objects (seeds of agricultural plants, animal spermatozoids, microalgae); studies in mechanisms of MMW interaction with plant cell as well as new MMW technologies and equipment for irradiating biological systems in a number of biotechnological processes (in plant growing, cattle breeding, in microalgae cultivation) have been presented.

Theoretical and experimental studies conducted in Russia, Ukraine and Israel during 30 years served as scientific foundation of biotechnologies mentioned. Series elements and blocks of microelectronics, radio engineering and mechanics serve as designing foundations of the devices developed. At present laboratory variants of several devices are available (e.g., a device for stimulating plant seeds growth, a device for activating animal spermatozoids, a device for microalgae biomass increase).

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