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Physical Factors for Plant Growth Stimulation Improve Food Quality

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1. Introduction

Fast growing of world population affected negatively the environmental conditions of our life. Increasing number of earth population resulted in growing consumption of food and energy. Both tendencies seriously exhaust the natural resources. The attempts to increase food and energy production for satisfying growing needs led to intensive development of plant production through the use of chemical additives, which in its turn caused more and more pollution of soil, water and air.

The environmental pollution cannot be limited in the region of the primary use of chemical additives. It spreads out horizontally - through the underground and surface waters it is passing out over the frontiers. Through the food chain including plants and animals it spreads out hierarchically and achieves our table. As a result the pollution negatively affects food quality.

The risk of food safety hazards occurring during on-farm production for fresh produce is closely related to the use of herbicides, fertilizers, and other chemicals. Soil and water contamination generates toxic compounds in plants that worsen the food quality. In order to raise food safety it is necessary to control and keep the concentration of harmful substances in foodstuff in reasonable limits. Even the biological agriculture hides danger of spreading infections like this one recently arisen with *Escherichia* Coli.

Chemical compounds are largely used for improving soil fertility; plant protection during growth and storage stage; for improving food quality at production, preservation and conservation stages; their application is partially allowed even in organic agriculture. Uncontrolled use of chemicals is hazardous for the contamination of raw materials for food production with toxins. Through the food chain the accumulation of different chemicals is dangerous for the health of consumers. Therefore, on-farm safety for fresh produce needs developing and implementing new methods for quality control and assurance. One possibility to reduce chemical contamination in raw materials is the substitution of chemical compounds with physical factors for plant growth stimulation.

2. Alternative technologies for increasing plant production

Nowadays, the diapason of agricultural practices lies between the swidden agriculture and high-level biotechnology. The use of physical factors takes interstitial position and represents interest for many scientists.

The use of physical factors for controlled influence on biological behaviour during development and storage of different cultures is a modern trend in combining the intensification of plant technologies with the ecological requirements. It could be important for biological and organic agriculture.

Physical methods for increasing the vegetable production are based on the use of different physical factors for plant treatment, particularly on the dill seeds. Most perspective factors include the treatment with electromagnetic waves, particularly optical emission, magnetic field as well as the ultrasound and ionizing radiation. All living processes are highly dependent on energy exchange between the cell and the environment. The core of physical methods is the energy supply through the treatment with physical factors.

Plants sensitivity to the influence of these physical factors has been elaborated during their evolutionary development since these physical factors are elements of their natural environment.

The influence of physical factors as microwave and laser radiation, magnetic field and ultrasound treatment is an alternative of soil additives and fertilizers. The substitution of chemical treatment by physical one can reduce toxins in raw materials and thus – raise the food safety.

Contemporary agriculture largely uses chemical compounds for improving soil fertility, plant production and protection against plant diseases and enemies.

The substitution of chemical amelioration with convenient physical methods of treatment has two advantages - one is reducing the use of fertilizers and thus decreasing pollution of on-farm produced raw materials for food production and the other – possibility for disinfection of seeds before sowing and during the storage through inactivation of microorganisms.

Physical factors might be good alternative of chemical products for the same purposes: raising the yield of agricultural production, improving plant protection and storage, as well as processing, preservation and conservation of food.

In the case of chemical amelioration the necessary substances are imported into the cell. Instead of this in the case of physical treatment the energy introduced in the cell creates conditions for molecular transformations and as a result, the necessary substances are provided for the cell.

2.1 Magnetic field

2.1.1 State of the art

One of most popular physical factors is magnetic field. Magnetic field is an attribute of the Earth. Its origin is the molten metallic core of our planet where circulating electric current appears. Magnetic field changes periodically its direction. Evidence for 171 magnetic field reversals during the past 71 million years has been reported. Its magnitude varies from Earth surface to the depth as well as over the surface of the Earth.

Numerous authors confirmed that a magnetic field of magnitude one or two orders above geomagnetic field strength (35 to 70 μT) could affect plant growth and metabolism (Galland & Pazur, 2005; Çelik et al., 2009; Racuciu 2011; Shine et al., 2011).

The impact of magnetic field on plants depends on its magnitude and its character – static or alternating. Different magnetic fields have been used for plant treatment – static (with constant magnetic induction), alternating (Aguilar et al., 2009), oscillating (Racuciu 2011), pulsed (Bilalis et al., 2011).

The positive influence of the stationary magnetic field on the plant seeds has been largely established (Galland & Pazur, 2005). The treatment fastens plants development (Florez et al. 2007; Gouda & Amer, 2009), improves germination and seedling growth (Carbonell et al., 2008; Martínez et al., 2009a; Odhiambo et al., 2009), activates protein formation and enzymes activity (Atak et al., 2007; Racuciu et al., 2007; Çelik et al., 2009). The investigations have shown that the treatment of the seeds with magnetic field increases the germination of non-standard seeds and improves their quality (Pietruszewski et al., 2007). Experiments have been made with large range of plants: grain - (Torres et al., 2008; Vashisth & Nagarajan, 2008; Vashisth & Nagarajan, 2010), leguminous (Podlesny et al., 2004; Podlesny et al., 2005; De Souza et al.; 2006, Odhiambo et al., 2009; Martínez et al., 2009b), and perennials (Çelik et al., 2008;, Dardeniz et al., 2006; Dhawi & Al-Khayri, 2009).

The effect of magnetic treatment on plants depends on the strength of magnetic field, expressed by magnetic induction, and the exposure time. There are significant differences in the induction B of investigated magnetic fields – from B = 62μT (Odhiambo et al., 2009) to 250 mT (Vashisth & Nagarajan, 2010). Explored exposure times also vary largely - from 15 s (Muszinski et al., 2009) to 24 hours (Martinez et al., 2009a).

Some authors have explored the influence of irrigation with preliminary magnetized water and also have found positive impact on plant development (Abdul Qados & Hozayn (2010a, 2011b); Hozayn et al. (2010, 2011); Hozayn & Abdul Qados (2010); Vajdehfar et al. (2011).

2.1.2 Materials and methods

In our last experiments the influence of static magnetic field has been studied on seeds of lentils (*Lens Culinaris*, Med.). The induction of used magnetic field has been B =0,15 T, measured with a digital Teslameter Systron - Donner. Seeds have been distributed in five variants and 5 replicate each one containing 10 seeds each. Seeds in each variant have been exposed to magnetic field treatment for different time: 0 min (control), 3 min, 6 min, 9 min, and 12 min.

Seeds have been preliminarily soaked in distilled water for 1 hour, presuming that the intra-cell water, due to its magnetic properties, plays role in the absorption of the energy of magnetic field. After the treatment the lentil seeds were cultured in small plastic pots (Ø =7.5 cm and h = 8.8 cm) on wet cotton. The natural light cycle was 9 h - light/15 h - darkness with daily temperature 21 ± 2°C, night temperature 15 ± 2°C.

In order to estimate the influence of the treatment on seeds in our experiments the next some criteria have been used hereafter:

- The germination energy (GE) of seeds in %, determined on the 4th day after the start of the experiment - as a ratio of the number of germinated to the total number of seeds for the corresponding variant;
- germination (G) of seeds in %, determined on 7^{th} (8^{th}) day as a ratio of the number of germinated to the total number of seeds;
- length of stems (SL) and main roots (RL) in mm determined on the 7^{th} and 14^{th} day;
- total mass (TM) in mg determined on the 14^{th} day.

Data were statistically processed using the method of Fisher of dispersion analysis. The reported values are mean values for 50 measurements of each parameter.

2.1.3 Results

The germination energy (GE) and the germination (G) are shown at Fig.1. Both parameters – GE and G, have the highest values for non-treated samples. The values for all variants are enough high – not less than 80%. Differences between variances and control are not statistically significant. This observation seems to be in contradiction with our earlier results for soybean (Aladjadjiyan 2003c) and ornamental trees (Aladjadjiyan 2003a), where a significant rise of germination has been accounted for treated samples. This contradiction may be due to the different kind of seeds used in previous experiments.

The results for the length of stem and root of lentils seeds measured on the 7^{th} day are similar - values for treated samples are lower than the control.

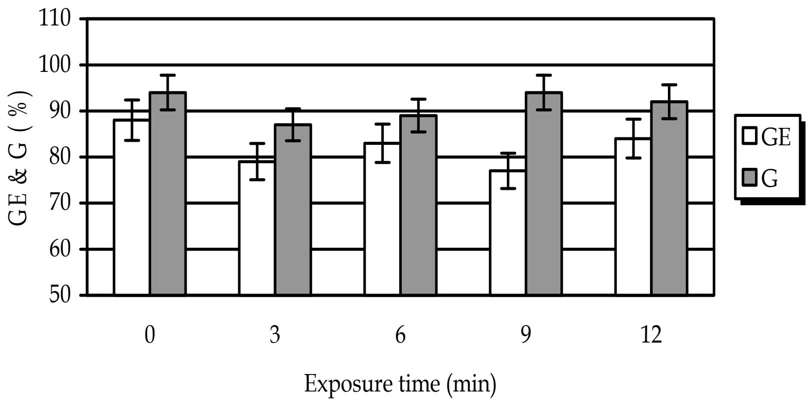

Fig. 1. Germination energy (GE) and germination (G) of lentils seeds exposed to static magnetic field.

The results for the measurements on the 14^{th} day show more expressive differences. The stem lengths shown on fig. 2 and the total mass on fig. 3 have well expressed increase for the samples treated with magnetic field for 6 and 9 min. In the case of 6 min exposure the increase of SL and RL is 120% and 11%, respectively; in the case of 9 min exposure - 104%

and 12%, for SL and RL, respectively. Longer exposure of 12 min results in SL and RL shorter than those for exposure 3 min (fig.2). Similar conclusions are valid for the total mass (fig.3). Values of TM for exposure 6 and 9 min are the highest, the value for exposure 9 min is even less than the control one.

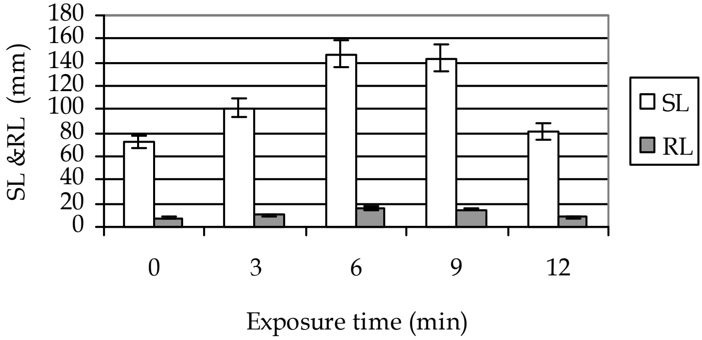

Fig. 2. Stem (SL) and root (RL) length of lentils seedlings measured at the 14th day.

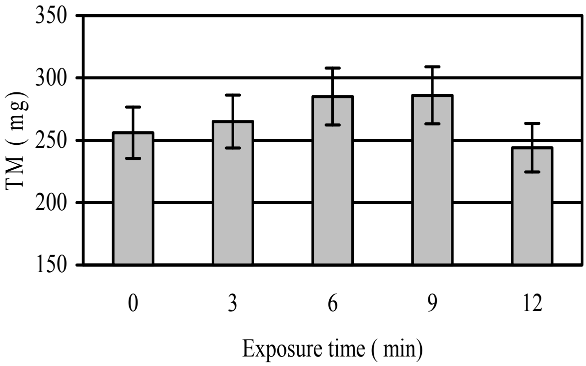

Fig. 3. Total mass (TM) of lentils seedlings exposed to static magnetic field at the 14th day.

The conclusion of our investigation is that for treatment of lentils seeds with stationary magnetic field with induction B=0,15 T the optimal exposure time is between 6 and 9 min. Our earlier experiments have shown that the best results for SL and TM in case of maize (Aladjadjiyan, 2002a) and soybean (Aladjadjiyan, 2003c) have been achieved for the same value of magnetic induction at 10 min exposure time, while in the case of *Nicotiana Tabacum* (Aladjadjiyan & Ylieva, 2003) a linear rise of G and GE in the interval 0 – 30 min has been registered. Similar results for maize have reported Hernandez et al., 2009. Their conclusion was that the impact of magnetic field treatment is most effective for the combination B=60mT and t=7,5 min, but depends also on seeds genotype. For higher intensity B=100mT the longer exposure time (30 min) leads to worse results. We have accounted the same for B=150mT and t=12 min.

2.1.4 Discussion

We tried to compare the effect of magnetic treatment in our investigation with those reported by different authors. We used the data of authors investigated the influence of static magnetic fields with value close to 0,15 T and exposure times ranging between 3 and 60 min. We calculated the maximum growth of germination (G), stem length (SL), and total mass (TM) in percentage towards the control. Data of Shine et al., 2011, and Flórez et al., 2007, have been used for producing additional data along with our own (Aladjadjiyan, 2002a, 2003a, b, c, 2010a) presented on fig.4. It seems that the influence of static magnetic field on the growth of investigated parameters G, SL, and TM reaches maximum for exposure time around 30 min. The investigation of Shine et al., 2011, on the treatment of soybean with magnetic field with intensity B=150mT showed the best results for all studied parameters at exposure 60 min. Compared to our results for soybean treated with stationary magnetic field with the same intensity it shows a difference – our best results for G have been achieved for exposure 15 min, and for stem length and total mass at 10 min (Aladjadjiyan 2003c), but we have investigated the interval of exposure times between 0 and 30 min. Muszynski et al., 2009, concluded that short exposure time (15 and 30s) does not influence wheat seedling growth.

The authors that have investigated longer exposure time (longer than 30 min) have accounted influence of the treatment on the concentration of chlorophyll *a* and *b* (Vashist & Nagarajan, 2008); mitotic activity (Racuciu, 2011); activities of superoxide dismutase and catalase (Celic et al., 2009). All these parameters have been significantly increased.

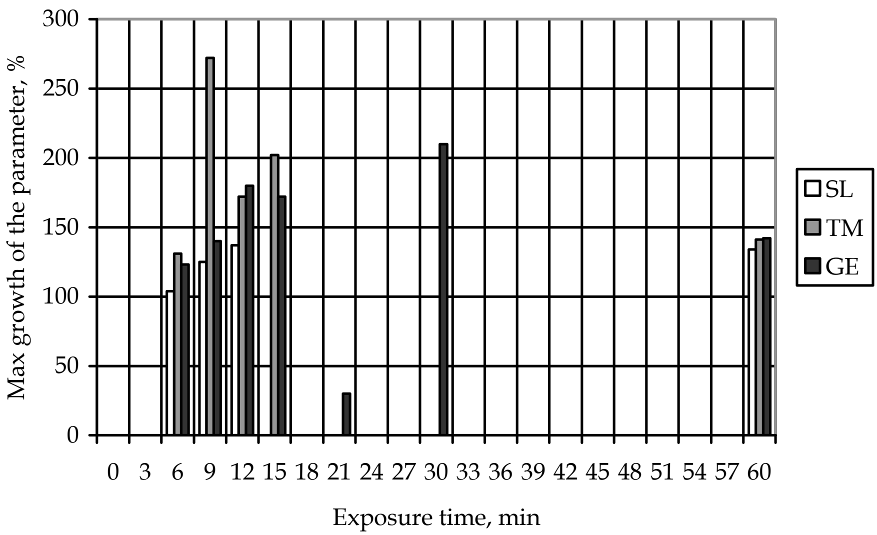

Fig. 4. The effect of static magnetic field on the growth of plant parameters.

This selective effect of different doses of magnetic field treatment may be explained with ions properties. Ions in the cell have the ability to absorb magnetic energy corresponding to specific parameters related to their vibration and rotation energy sublevels. This phenomenon represents a kind of resonance absorption and could explain the stronger effect of applying definite values of magnetic field induction, observed as well in the works of Martinez et al., 2009.

One of the possible explanation of observed positive effect of magnetic treatment could be found in paramagnetic properties of some atoms in plant cells and pigments, i.e. chloroplasts. In outer magnetic field magnetic moments of these atoms turn align the field. Magnetic properties of molecules determine their ability to absorb the energy of magnetic field, then transform it in other kind of energy and transfer this energy later to other structures in plant cells, thus activating them. Magnetic effects on plants can be explained in the framework of the ion cyclotron-resonance and the radical pair models, two mechanisms that also play an essential role in the magnetoreception of other organisms (Galland & Pazur 2005). The hypothesis proposed by Shine et al., 2011, underlines the role of Ca^{2+} ions. The increased concentration of Ca^{2+} ions after the treatment possibly plays the role of signal for cells to enter earlier in mitotic cycle. Magnetic field affects also the electroconductivity (Szczez et al., 2011) thus changing cell status.

2.2 Laser

2.2.1 State of the art

Light is most important attribute of natural environment for life. The sensibility of plants to light is well known. Light is necessary condition for photosynthesis – most important process in plants' life. Plants' sensor for light is chlorophyll. In chlorophyll centrums the light energy is being transformed and used for producing carbohydrates. Plants are sensitive to light intensity, its spectral composition and alternating of light and dark periods.

Laser (Light Amplification by Stimulated Emission of Radiation) is specific light source. The light emitted by laser is notable for its high degree of spatial and temporal coherence, unattainable using other technologies. Laser light is uniformly polarized and monochromatic.

A detailed review of the use of laser treatment for plant stimulation has been published recently by Hernandez et al., 2010. As it is underlined there, laser light is used in agriculture for biostimulation of seeds, seedlings and plants on the basis of the synergism between the polarized monochromatic laser beam and the photoreceptors absorbing it, which activate numerous biological reactions.

Lasers used in agriculture belong to different types – solid, gaseous, semiconductor (depending on the state of emitting medium). Their emission also differs – from red light emitted by ruby laser (694 nm), and helium-neon (632,8 nm); green light emitted by YAG:Nd laser (532 nm); blue - by argon (514,5 nm); ultraviolet emission by nitrogen (337,1 nm); visible and infrared diapason covered by semiconductors (510, 632, 650, 670, 810, 940, and 980 nm), and carbon dioxide in far infrared (10 600 nm). A laser can be classified as operating in either continuous or pulsed mode, depending on whether the power output is essentially continuous over time or whether its output takes the form of pulses of light on one or another time scale.

The effect of laser treatment depends on the wave length of its emission, but also on the output power of the laser and the exposure time. The investigated output powers vary in large diapason between 200 kW (Govil et al., 1991, as cited in Hernandez et al., 2010) and 5 mW (Grygierzec, 2008 as cited in Hernandez et al., 2010). The exposure times as well vary largely – between 30 s used by Michtchenko & Hernández, 2011, and 120 min in the work of Khalifa &. El Ghandoor, 2011.

In Bulgaria the treatment with helium-neon laser was most spread-out (Aladjadjiyan 2008) probably because its relatively low cost but highly coherent emission. Due to the impact of helium-neon laser, an acceleration of germination and development at the early phases for dill seeds of different cultures was established. It was found out that the effect of stimulation depended on the laser wave length, the exposition on irradiation, the reiteration and the pre-history of the samples (i.e. preliminary soaking of seeds in water).

2.2.2 Materials and methods

We have investigated the influence of impulse and continuous helium-neon laser on the development of some plants. An impulse laser with wave length 632,8 *nm* and intensity 100 Wm^{-2} was used for irradiation of seeds of carrot (*Daucus carrota* L., cv.Nantes). The light-impulse's durability was 1 min. Three variants of treatment have been experienced: 5-, 7- and 9-fold irradiation. To assess the effect of the treatment, the germination energy GE, the germination G, and the total mass TM on the 7th day had been measured as described in previous part 2.1.2.

The influence of laser treatment has been investigated also on bean (*Phaseolus vulgaris* L., cv. Plovdiv) seeds. In this case continuously emitting He-Ne laser with intensity 176 Wm^{-2} has been used. Seeds have been distributed in four variants. Each variant has been exposed to laser treatment for different time: 0 min (control), 5 min, 10 min, and 15 min. In this case the germination energy GE, the germination G, and the total mass TM on the 38th, 45th, and 52nd day had been measured according to description in part 2.1.2.

2.2.3 Results

The results of the treatment of carrot seeds with an impulse He-Ne laser are presented for G and GE on fig.5, and for TM – on fig.6, respectively. From fig. 5 it can be seen that the best results for GE of carrot seeds have been achieved in case of 5-fold (total accumulated exposure 5 min) treatment with He-Ne laser; for G - in case of 7-fold treatment. The differences for both values are not statistically significant.

The 9-fold treatment (total accumulated exposure 9 min) has shown an inhibitory impact on GE and G, which could be due to an excess of light energy absorbed by the seeds in this configuration.

Figure 6 shows the dependence of the total mass (TM) on the exposure time. It can be seen that the best result for TM is accounted for 9-fold treatment. In all cases for described experimental configuration the differences are not statistically significant, except of the 9-fold irradiation. Possible explanation of this observation is that the combination of laser light intensity and the exposure of seeds for more cases do not assure enough energy for demonstration of stimulating effect.

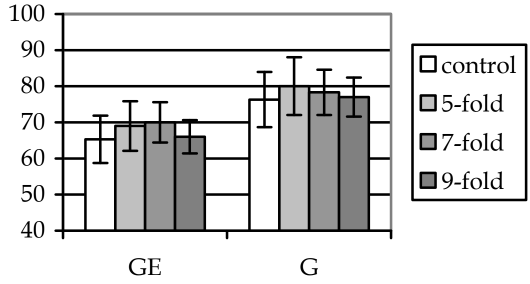

Fig. 5. Germination energy (GE) and germination (G) of carrot seeds treated with laser.

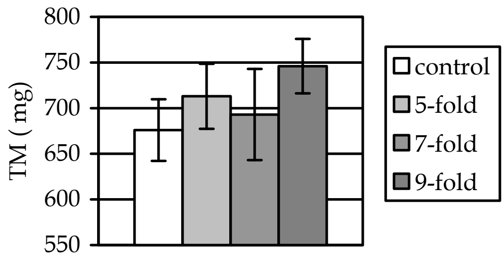

Fig. 6. Total mass (TM) of carrot seedlings after laser treatment, measured at the 7th day.

The results for the treatment of bean seeds with a continuously emitting He-Ne laser are presented on fig. 7. The stem length of 50 seedlings has been measured on different days

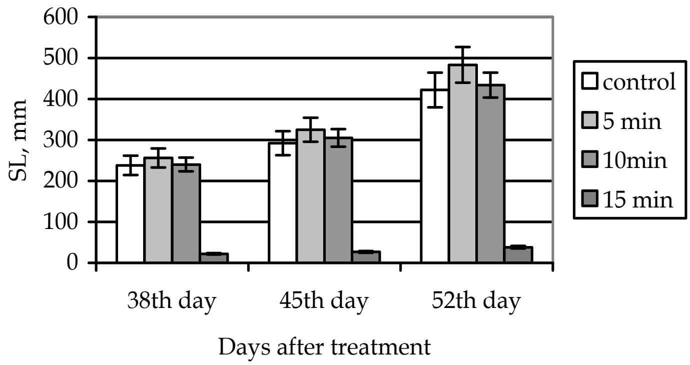

Fig. 7. Stem length (SL) of bean seedlings treated with He-Ne laser.

after treatment. It can be seen that the best result for this experimental configuration has been achieved for 5 min laser treatment. Longer laser treatment acts as an inhibitor. In the second configuration the intensity of laser emission is almost twice as strong as in the previous experiment. Exposure of seeds is equal (5 min) and longer than in the previous experiment. The combination of the higher light intensity with exposure time longer than 5 min probably introduce too much energy in the cell and instead of stimulation leads to inhibition of plant growth. We found that in this case the preliminary soaking of seeds in water showed worse results than those for dry seeds (Svetleva & Aladjadjiyan, 1996). In opposite - for magnetic field treatment the preliminary soaking improves the effect.

2.2.4 Discussion

Our results for the treatment with impulse He-Ne laser have shown the highest germination for the 5- and 7-fold irradiation. It can be compared with the results reported in the paper of Cwintal et al., 2010. Pre-sowing stimulation of seeds of alfalfa with He-Ne laser beam at intensity 6 mW/cm^2 (i.e. 60 Wm^{-2}) in their experiments has had a highest result for 3-fold and 5-fold exposure - it caused a significant increase in the content of specific protein, phosphorus and molybdenum in dry matter of the plants, and a decrease in the content of crude fibre.

Possible explanation of the inhibitory effect of preliminary soaking of seeds in water, observed in our investigation (Svetleva & Aladjadjiyan, 1996) could be related to re-distribution of energy emitted by laser. In the soaked seeds part of energy probably is absorbed by the imbibed water and the chlorophyll centrums received less energy compared to dry seeds.The explanation of these effects of laser treatment is not consistent. Some authors, as sited in Hernandez et al., 2010, accept that laser treatment can be regarded as a stress agent damaging cells and tissues. Salyaev et al., 2007, (as cited in Hernandez et al., 2010) suggest that a general cell response induced by laser light irradiation can be divided into two specific responses: the first one consists in a rapid stress effect resulting in an increase in the amount of lipid peroxidation products, and the second and longer one include the secondary reactions related to the adaptive metabolic changes and apparently accompanied by the stimulation of morphogenetic processes.

Numerous studies, cited in Hernandez et al., 2010, show a positive effects of pre-sowing laser irradiation, both on cereals (like rice, maize), and vegetables (tomato, radish, peas, cucumber, lettuce, onion, etc.). Several studies report that the seeds of vegetables are more sensitive and susceptible to laser stimulation than cereals (Drozd & Szasjener, 1999, and Gladyszewska, 2006, as cited in Hernandez et al., 2010). In opposite, the investigation of Yamazaki et al., 2002, as cited in Hernandez et al., 2010, showed 36% decrease in number of tiller spikes and 60% decrease in seed yield at final harvest of rice, treated with red-laser diode supplemented with blue light. The authors suggest the necessity of an optimization process when laser is applied as a pre-sowing treatment. Our experiments also support their opinion.

2.3 Ultrasound

2.3.1 State of the art

The sensibility of plants to ultrasound is related to mechanoperception (Telewski, 2006). Plants live in an environment including influence of mechanical forces – gravity, pressure of different flows (atmospheric, water), and have developed sensing of mechanical signals.

Ultrasound is a mechanical wave having frequency higher than 20 kHz. It was established that the treatment with ultrasound could change the state of the substances and even accelerate the reactions (Suslick, 1994). This fact motivated its application for stimulating the growth of different cultures. Sonification has been found favourable for the acceleration of early stages of plant development.

Ultrasound has been shown to have strong effect on plants, particularly on seed germination (Davidov, 1961; Timonin, 1966; Halstead & Vicario, 1969; Hageseth, 1974; Weinberger & Burton, 1981; Miyoshi & Mii, 1988 as cited in Telewski, 2006). Timonin, 1966, as cited in Telewski, 2006, reported that ultrasound treatment altered the viscosity of macromolecule solutions in seeds.

Ultrasound treatment of seeds often was used for industrial purposes like oil extraction and malts preparation (Kobus 2008; Tys et al, 2003), as well as for seeds' disinfection (Nagy et al. 1987; Nagy 1987). The obtained results are caused by cell destruction under the shock of the mechanical wave with high intensity. Cell destruction facilitates oil extraction; it also destroys infection transmitters.

Another application of ultrasound treatment for stimulating plant growth is also investigated. Different cultures were subjected to ultrasonic stimulation: pepper, tomatoes and cucumbers (Markov et al. 1987), fodder beans (Rubtsova 1967), radish (Shimomura, 1990), corn (Hebling et al. 1995), carrot (Aladjadjiyan 2002b), chickpea, wheat, pepper, and watermelon (Goussous et al. 2010), ornamental trees (Aladjadjiyan 2003a, 2003b), barley (Yaldagard et al. 2008a, 2008b, 2008c).

Obtained results have indicated that the effects of the treatment on seed germination depend on frequency of ultrasonic wave and exposure time as well as on plant species and cultivars. Most of the authors recommended the treatment with ultrasound of frequencies 15 – 100 kHz and exposition from 1 to 60 min, with radiation density between 1 and 10 Wcm^{-2} .

2.3.2 Materials and methods

In our previous works the effect of ultrasound treatment with a frequency of 22 kHz and a power of 150 W on the germinating energy and germination of carrot seeds (*Daucus carota* L.), cv. Nantes was studied (Aladjadjiyan 2002b). The maximum effect was established for 5 min treatment. Similar results have been obtained for some ornamental species (Aladjadjiyan 2003a, 2003b).

To explore the role of different parameters later the ultrasonic treatment of seeds has been implemented with an apparatus Carrera Sinus 2501 with frequency 42 kHz and power 100 W. In this investigation seeds of lentils (*Lens Culinaris*, Med.) and wheat (*Triticum aestivum*) have been used (Aladjadjiyan 2011).

From the theory of acoustics it is known that the intensity I of ultrasonic wave is related to its frequency ω:

$$I = \frac{\rho \omega^2 A^2}{2} v \tag{1}$$

where ρ is the density of the medium, A is the amplitude of the ultrasonic wave, and v - the velocity of the sound.

The bigger frequency means bigger intensity of ultrasound wave and a stronger influence on the samples. That is because for treatment of lentils and wheat seeds shorter exposure times were chosen compared with those in the case of carrot seeds and ornamental trees (Aladjadjiyan, 2002b; Aladjadjiyan, 2003a, b).

Seeds have been distributed in four variants, respectively for lentils and wheat. Each variant has been repeated in 10 replicates containing 10 seeds each. Seeds in each variant have been exposed to ultrasound for different time: 0 min (control), 1 min, 2 min, and 3 min. Seeds have been soaked in plastic containers with tap water and placed in the centre of the ultrasonic apparatus. After the treatment the seeds were cultured in small plastic pots (Ø =7 cm and h = 7 cm) on wet filer paper.

To assess the effect of the treatment, the germination energy GE, the germination G, stem (SL) and root (RL) length and the total mass TM on the 7th and 14th day had been measured as described previously in part 2.1.2.

2.3.3 Results

The results for the SL and RL for seeds of lentils and wheat measured on the 7th day are illustrated on fig.8. It can be seen that the values for treated samples both for SL and RL rise with exposure time. The rise of SL for lentils at exposure time 1 min is 24%, for 2 min - 86%, and for 3 min – 100%. In case of wheat seeds the rise of SL is not so strong. It is respectively 9% for 1 min, 5% for 2 min and 16% for 3 min exposure. The rise of RL both for wheat and lentils seeds is well expressed. The values for lentils are 67% for 1 min exposure time, 130% for 2 min and 214% for 3 min. In the case of wheat it is 56%, 122 and 148%, respectively.

The dependence of SL and RL *vs*. exposure time in the case of lentils is approximately linear, while in the case of SL for wheat it is weak and the differences are not statistically significant.

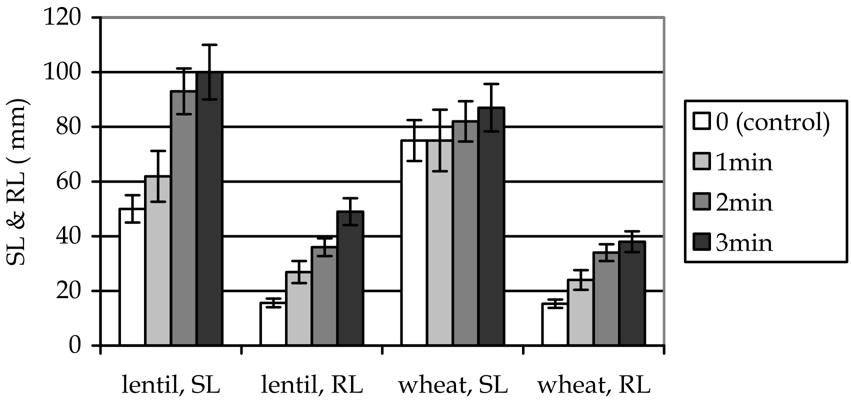

Fig. 8. Stem (SL) and root (RL) length of sonified lentils and wheat seeds at 7th day

The results for the measurements on the 14th day show some differences between the behaviour of lentils and wheat seeds. In the case of lentils SL (fig.9) for treated samples are longer than the control one. The change is respectively 35% for 1 min, 18% for 2 min and 18,5% for 3 min. A well expressed maximum is accounted for exposure time 1 min. In the case of wheat the longest SL is measured for untreated (control) samples. The values of SL for treated samples are less than control one. The values of RL for samples treated at exposure time 1 min are bigger than the control, both for lentils and wheat. The rises are 7% and 10% at exposure time 1 min, for lentils and wheat, respectively. In the case of wheat the difference is statistically significant. For exposure time 3 min the rise 6% has been accounted only for lentils.

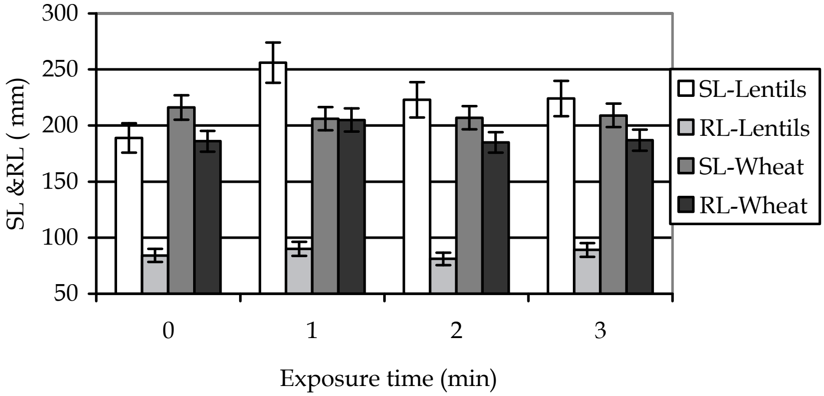

Fig. 9. Stem (SL) and root (RL) length of sonified lentils and wheat seeds at 14th day.

It have to be pointed out that only the length of the main root has been measured without taking into account the lateral roots. This can partially explain the differences in the rise of TM for the samples, for which a rise of SL and RL was not accounted.

The dependence of the total mass of lentils and wheat seeds *vs.* exposure time is presented on fig. 10. It can be noticed that both for lentils and wheat the TM of all the treated samples

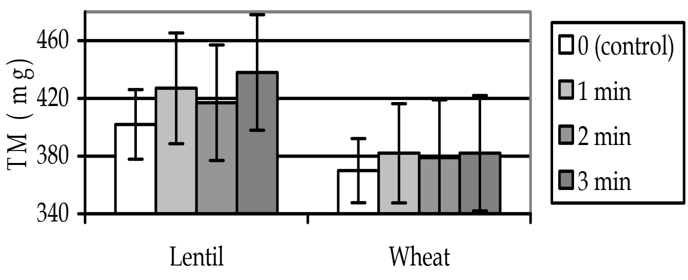

Fig. 10. Total mass (TM) of sonified lentils and wheat seeds at 14th day.

are bigger than control ones. The comparison shows that the TM of the samples of lentils are bigger than those of wheat, but in the case of wheat the difference between TM of treated and control samples is smaller than that for lentils. For all the variants the differences are not statistically significant. The rise of TM is 6%, 4%, and 9% for exposure times 1min, 2min, and 3min, respectively, in the case of lentils. In the case of wheat it is 3%, 2%, and 4%, respectively. The differences are not statistically significant.

2.3.4 Discussion

Suslick (1994) mentioned that the chemical effects of ultrasound are diverse and include substantial improvements in chemical reactions. In some cases, ultrasonic irradiation can increase reactivity by million times. The rise in plant growth characteristics for both lentils and wheat, better expressed for the measurements on the 7th day, may be explained with the increased reactivity of biological substances in the seeds under the influence of ultrasound. Later measurements on the 14th day show that the differences between the characteristics of treated and control samples decrease. This fact may be attributed to the kinetics of the effect of ultrasonic treatment on chemical reactions - possibly occurs some attenuation of this effect in the time.

2.4 Microwave radiation

2.4.1 State of the art

The microwave radiation is electromagnetic radiation with frequencies between 0,3GHz and 300GHz. Most investigated is the radiation 2,45 GHz because it is absorbed by water molecules, present in all live cells.

Banik et al., (2003) reviewed the bioeffects of microwave, mostly on animal and human health. In their paper the most popular opinion has been outlined, that the effect of microwave is attributed mainly to the heating. Nevertheless it has been mentioned that there are also non-thermal microwave effects in terms of energy required to produce molecular transformations.

It has been accepted (Buffler, 1993), that the thermal effect of microwave is related to the interaction with charged particles and polar molecules. Microwave fields are a form of electromagnetic energy and its interaction with charged particles and polar molecules leads to their agitation which is defined as heat. Biological material placed in such radiation absorbs an amount of energy which depends on the dielectric characteristics of the material. The thermal effect of electromagnetic fields from radiofrequency diapason on biological objects is evaluated by Specific Absorption Rate (SAR), defined as the power absorbed per mass of tissue and measured in Wkg^{-1}. The use of SAR for assessment of microwave impact is reported in literature for different biological objects but not for seeds.

In most of the published investigations concerning agriculture the microwave treatment has been used for disinfection of seeds before sowing. Bhaskara Reddy et al. 1995, 1998 used successfully the treatment with electromagnetic radiation from the radio- (10 - 40 MHz) and microwave diapason (2,45 GHz) on seeds of mustard, wheat, soybean, peas and rice seeking to eliminate the microorganisms (*Fusarium graminearum*) before seed storage. Similar aims have been described in the PhD thesis of V. Rajagopal, 2009. He has treated grain seeds with microwave radiation aiming disinfection, too. In his work a pilot-scale industrial microwave dryer operating at 2,45 GHz was used to determine the mortality of life stages of *Tribolium castaneum* (Herbst), *Sitophilus granarius* (L.) and *Cryptolestes ferrugineus* (Stephens) adults in wheat, barley, and rye. In the listed works there were no data about SAR evaluation. Tylkowska et al., 2010 have investigated the influence of treatment with microwave radiation (2,45 GHz) with output power 650 W and exposure times between 15 and 120 s on bean seeds infected with 13 fungi species and have found decreasing of infection and increasing of seed germination.

Some authors have investigated the influence of microwave treatment on different properties of seeds. Yoshida et al., 2000 treated soybean seeds with microwave radiation (2,45 GHz) for 6 to 12 min with the aim to improve the distribution of triglycerides in the seed coat. Oprică, 2008 has studied microwave treatment with power density under 1 mW/cm^3 on rapeseeds (*Brassica Napus*) and concluded that the microwaves determined variations of catalase and peroxidase activities depending on the age of the plants, time of exposure and state of seeds (germinated and non germinated) exposed to microwave. In all above-mentioned studies the microwave treatment was oriented to produce effects not related to plant stimulation.

Ponomarev et al., 1996 have investigated the influence of low intensity microwave radiation on the germination of cereals (winter and spring wheat, spring barley, and oats). Radiation with wavelength λ = 1 cm at exposition up to 40 min was used. An increasing of germination for all the treated seeds was observed, the optimum effect of stimulation being accounted at the exposition for 20 min.

Jakubowski (2010) has examined the impact of microwave radiation at frequencies ranging within (2.45-54) GHz on selected potato plant life processes and has found positive impact of microwave radiation at frequency 2.45 GHz on the weight of irradiated seed potato germs, and tubers. For the other investigated frequencies no positive results were accounted.

The treatment with microwave radiation as a stimulation agent in agriculture is not enough investigated yet. The stimulation effect of microwave treatment has been investigated by Aladjadjiyan & Svetleva, 1997 on bean (*Phaseolus vulgaris*) and on some ornamental perennial species *Caragana arborescens Lam.*, *Robinia pseudoacacia L.*, *Gleditsia triacanthos* and *Laburnum anagyroides* Med. (Aladjadjiyan, 2002a) and some encouraging results have been established.

The stimulation effect of microwave treatment for longer exposure time and higher irradiation power by investigating its influence on the early stage development of lentil seeds (*Lens culinaris*, Med.) has been performed by Aladjadjiyan, 2010.

2.4.2 Materials and methods

In our experiments the influence of microwave irradiation with wave-length 12 cm on seeds of lentil (*Lens culinaris,* Med) has been investigated. A magnetron OM75P(31) with frequency of radiation 2,45 GHz and maximum output power 900 W according to supplier's data has been used as microwave source. Maximum density of irradiation has been estimated at 45 kW/m^3. The estimation has been obtained by dividing the output power of the device (900 W) to the working volume having dimensions 0,19x0,33x0,32 m^3.

In earlier investigation (Aladjadjiyan & Svetleva, 1997) we have found that preliminary soaking of seeds in distilled water increased the effect of stimulation by more than 25 % due to the specific absorption of microwave radiation with wavelength of λ=12 cm by water molecules. That is because lentils seeds have been preliminarily soaked in distilled water for 1 hour, presuming that the imbibed water plays an important role in the absorption of the energy of microwave radiation.

Seeds for the experiment have been distributed in five variants and 5 replicates each containing 10 seeds. The variants differ by the time of exposure to the microwave radiation. Seeds have been exposed to the microwave radiation for 0 s (control), 30 s, 60 s, 90 s, 120 s. Two modifications of output powers of magnetron – 450 W and 730 W, corresponding to intensities - 22,5 kW/m^3 and 36,5 kW/m^3 respectively, have been applied.

2.4.3 Results

The effect of microwave treatment of lentils seeds on the germination energy GE and germination G s presented on fig.11, on SL and RL measured at the 7th and 14th day of sowing– on fig.11 and fig.12, respectively. Total mass of seedlings measured at the 14th day is presented on fig.13.

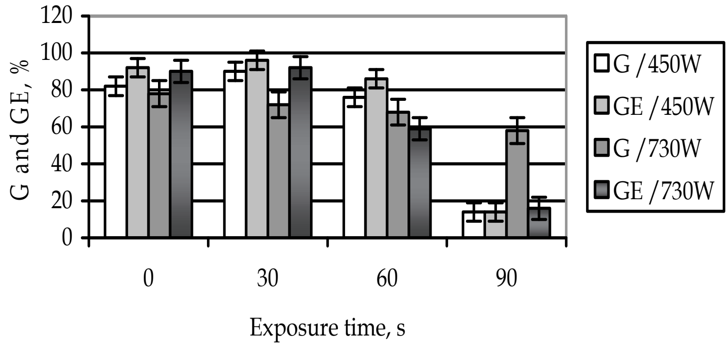

Fig. 11. Germination G and germination energy GE for lentils seeds treated with microwave for different exposure time (0, 30, 60, and 90 s) and output power (450 & 730W).

It can be noticed from fig.11 that for microwave treatment with output power 450 W the highest results for GE and G have been obtained for the exposure time 30 s. This exposure time has shown stimulation effect. All data were significantly different from control. For irradiated samples GE has risen with 9,8 %, while G – with 4,3 %.

The microwave treatment with output power 730 W shows that as well as in the case of treatment with 450 W, the values of G also demonstrate an effect of stimulation for the

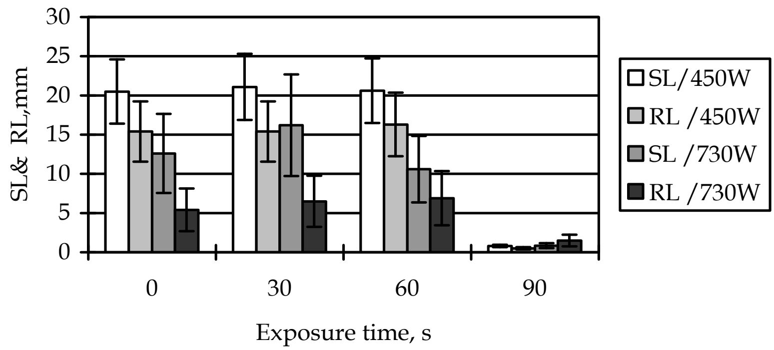

Fig. 12. Length of stems (SL) and roots (RL) for lentils seeds on the 7th day.

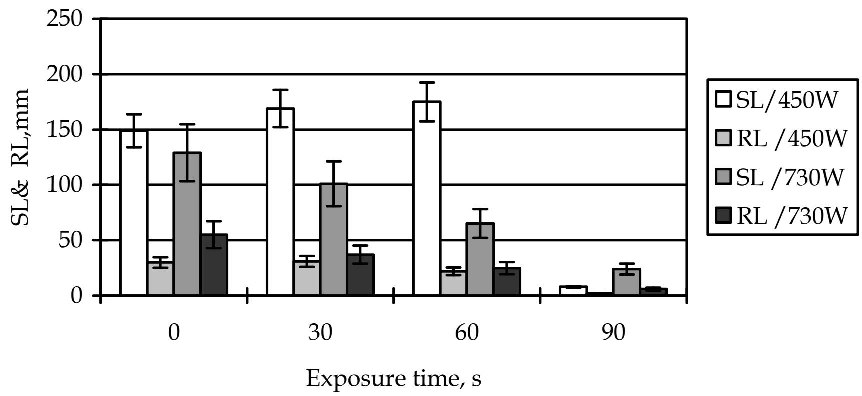

Fig. 13. Length of stems (SL) and roots (RL) for lentils measured on the 14th day.

exposure time 30 s. The differences for GE at exposure 30 s and G at exposure 60 s from the control are not significant. An inhibition of GE can be accounted for longer exposure time (60 and 90 s) as well as for G at exposure 90 s.

The comparison of data for 450 W and 730 W allows concluding that the positive effect of treatment generally is stronger for the lower output power of microwave irradiation – 450 W. Shorter exposure time (30 s) demonstrates higher stimulation effect than longer ones. Exposure time 120 s causes total inhibition.

It have to be pointed out that only the length of the main root has been measured without taking into account the lateral roots. This can partially explain the differences in the rise of TM for the samples, for which a rise of SL and RL was not accounted.

It have to be pointed out that only the length of the main root has been measured without taking into account the lateral roots. This can partially explain the differences in the rise of TM for the samples, for which a rise of SL and RL was not accounted.

The image on fig.13 shows that SL has higher values for the plants treated with microwaves with power 450 W than those for 730 W. The positive effect is accounted for the exposure times of 30 and 60 s. For the treatment with power 450 W at exposure 30 s the value of SL is 12,5% longer than the control one and for exposure 60 s the SL is 13,7% longer. For the treatment with power 730 W the values of SL are shorter than the control. All the differences are statistically significant.

The total mass (Fig.14) of plants *vs.* exposure time rises linearly for the treatment with microwave power 450 W from 0 to 60s, while for the one treated with 730 W there is a maximum at exposure time 30 s. Longer exposure times for the configuration with power 730 W demonstrate an inhibitory impact on total mass values.

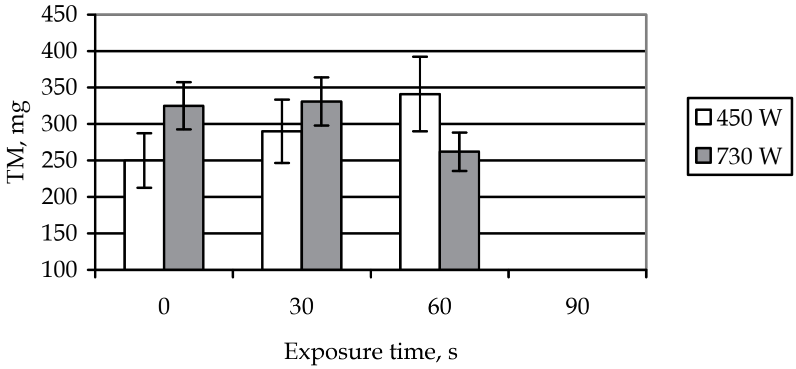

Fig. 14. The total mass (TM) of lentils seeds measured on the 14th day.

2.4.4 Discussion

Total mass for the samples treated with 430 W at 30 s is 16% higher, and for those at 60 s TM is 36,4 % higher than the control. One can conclude that for 450 W the exposure time 60 s is more effective in later stages of development than the exposure at 30 s. A controversy with the data about RL for the same configuration could be noticed. The results on fig.12 show that at exposure 30 s root length is 3% longer, but at exposure 60 s it is 30 % shorter than the control. This controversy could be attributed to the fact that the RL only of the main root is measured; but there are lateral roots that contribute to the weight and are not accounted for root length. This explanation refers also for the accounted rise of TM with 5% for the samples, treated with 730 W at exposure 30 s.

Compared to other examined methods microwave is considered as most harmful one. There are investigations contending the negative influence of microwave treatment on plant development - Jangid et al. 2010 have found that the treatment with microwave oven 2,45

GHz, 800 Wcm^{-2} for 7 s induces mutations in seedlings of moth bean [*Vigna aconitifolia* (Jacq.) Marechal]. The authors have found positive influence for shorter exposure time (1, 3, and 5s). Our observations are similar – longer exposure time inhibits the development of seedlings. On the basis of these experiments the suggestion maybe formulated to use cautiously the treatment with microwave and shorter exposure times to be chosen.

3. Conclusion

The treatment with physical factors like different kind electromagnetic fields as well as ultrasound improves germination and early stages of development of plant seeds.

Correct application of physical methods of stimulation requires preliminary experimental investigation and establishment of convenient regimes, which for all the studied cases strongly depends on plant characteristics, intensity of physical factor and exposure time.

Experimental investigations of the physical factors' influence on plant development may help to clarify the mechanisms of energy exchange in molecules and thus stimulation of plant development.

The substitution of chemical methods of plant growth stimulation with physical ones can help avoiding the pollution of food raw materials with toxic substances.

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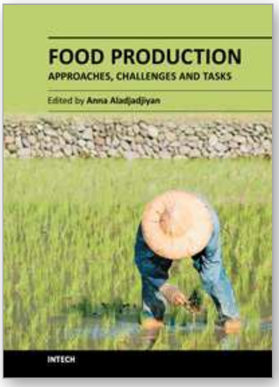

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This book is devoted to food production and the problems associated with the satisfaction of food needs in different parts of the world. The emerging food crisis calls for development of sustainable food production, and the quality and safety of the food produced should be guaranteed. The book contains thirteen chapters and is divided into two sections. The first section is related to social issues rising from food insufficiency in the third world countries, and is titled "Sustainable food production: Case studies". The case studies of semi-arid Africa, Caribbean and Jamaica, Burkina Faso, Nigeria, Pacific Islands, Mexico and Brazil are discussed. The second section, titled "Scientific Methods for Improving Food Quality and Safety", covers the methods for control and avoidance of food contaminants. Substitution of chemical treatment with physical, rapid analytical methods for control of contaminants, problems in animal husbandry related to diary production and hormones in food producing animals, approaches and tasks in maize and rice production are in the covered by 6 chapters in this section.