

Testing the effect induced by the simultaneous treatment with magnetic field and laser light field, modulated at audio frequencies, on colonies of *Alternaria alternata*

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Abstract

The reaction capacity of imperfect *Alternaria alternata* fungi to the action of the magnetic field and of the laser light one, modulated at audio frequencies, was analyzed by executing several experimental variants, selecting the optimum variants and performing a number of 3 rehearsals with the selected variants for each sample.

Since the objective proposed by these researchers was that of executing comparative laboratory tests for highlighting the effect produced by the simultaneous irradiation with magnetic field and laser light field, modulated at audio frequency, on types of pathogens in plants, the observations were focused on the performing of determinations of the inactivation and inhibition percentage of their development.

The use of laser diodes having the radiation emitted, of red colour, for treating cultures, for the purpose of increasing the inactivation and inhibition percentage, the experiments performed, have highlighted the fact that it is important to establish the dose of irradiation with laser light, modulated at audio frequencies, for each separate variant. From the experiments initiated it was noticed that the two types, **A1** and **A100** from the colonies of microbial cultures from the imperfect fungi groups *Alternaria alternata* are influenced by the treatments with red light and by the magnetic field waves applied simultaneously, the intensity of the answer being conditioned by the biological particularities of the biological material and by the irradiation doses.

Key words: laser diodes, magnetic field, modulation, inactivation, fungi.

Introduction

Intensive agriculture and the present requirements, but, especially, the prospective requirements in the field, give special importance to all factors contributing to the increase of production, a special place being occupied by the protection of cultures against pathogenic agents and pests [1, 2 and 3].

The phytosanitary protection of vegetable cultures, with all aspects regarding the efficacy of treatments, the reduction of environmental pollution, etc., constitutes at present, as well, a research activity field in support of vegetable production. Generally, following giving up the chemical fight against illnesses, the pests and weeds, both the quantity and the quality of the agricultural production suffer. The uncontrolled propagation of pests, illnesses and weeds causes the intoxication and contamination of food products with toxins, "natural", it is true, but no less dangerous than the synthesis one. Intoxications due to impurities, which lead to the contamination of agricultural products with mycotoxins, following by the attack with different pathogenic agents from the types of *Aspergillus* and *Fusarium* are known [4]. Their action may be graded from chronic to acute toxicity until cancer-producing effects.

In plant therapy were used some different low power monochromatic radiations. These kind of non conventional treatments were subject of some national and international research projects. The results show their benefit in protection process of plant. Using of the audio modulated laser radiation and magnetic fields treatment shows a significant influence on plants growth [5,6,7].

The present research has as purpose the design and the obtaining of the certain ecological technologies on basis of the inhibition of development of pathogenic fungi colonies in the culture of *Solanaceae* plants (tomatoes, peppers and eggplants) by irradiation with laser light, modulated in audio frequencies and magnetic field, with significant results on the qualitative and quantitative improvement of the vegetable production, but also on the reduction of the degree of pollution of production and environment, by decreasing the use of chemical substances. The purpose of the research performed is to find a method which can inhibit the increase and proliferation of *Alternaria alternata* colonies cultivated *in vitro* by the simultaneous treatment with magnetic field and laser light, modulated at audio frequencies.

Material and Method

Biological material

Biological material consists on microbial *Alternaria alternata* cultures, **A1** and **A100** types, isolated from tomatoes and cultivated on the PDA type culture medium produced by Fluka (potato-dextrose - agar with: potato extract 4g/L, dextrose 20g/L and agar 15g/L, ph=5.4) taken from the collection of the Genetics Laboratory of the Faculty of Biotechnologies (USAMV-Bucharest). For preparing one litre of medium there were used 39g of PDA powder in 1000ml distilled water. After homogenization, the medium was sterilized at 121°C, for minim 20 minutes [1, 2 and 4].

Manner of work used in establishing microbial cultures

As materials were used: Petri plates ø10 cm, automated pipette of different capacities, test tubes with sterile distilled water, microbial cultures of *Alternaria alternata* from the **A1** and **A100** types. For each experimental variant were executed 3 rehearsals (plates).

After sowing, the plates were thermal preserved at 25-26°C for 3 days, and then they were simultaneously treated with magnetic fields and red colour laser light. All 3 Petri plates considered rehearsals, prepared for each experimental variant, were irradiated for 5 days, and the measurements and observations were reported to the control culture not irradiated, also prepared in 3 rehearsals for each of the 2 *Alternaria alternata* types.

Method. Experimental device

The experimental device is built out of an irradiation device with laser light coming from laser diodes with emission in the red spectral field and device for generating the homogenous magnetic field.

a) The experimental device used for the irradiation of *in vitro* cultures in magnetic field is made up of a source that generates a magnetic field at a frequency defined a priori by a bipolar amplifier that enhances the signal created with sources of arbitrary functions and one solenoid. The values of the magnetic field in which plants and microbial cultures answer favorably are under 50 mT, and their frequencies are close to the network frequency of 50-60Hz. Inside the device, where the magnetic field is homogenous, there is placed the support on which the bowls that must be irradiated (1 Petri bowl with 1 microbial culture /variant of treated sample) can be exposed on. The energy emitted by the device is allotted in a volume of 20x20x20cm³. The selection of the exposure time was performed in accordance to the irradiation with laser light field and with the desired irradiation dose.

b) The experimental device with laser diodes with emission in the spectral field 640-660nm and intensity modulated in audio frequencies in the range (5Hz÷20kHz) is made up of laser head, gripping system “mobile arm” type and the supply source which also comprises the system of modulation in audio frequency.

The laser head is made up of 19 laser diodes aligned inside a cylinder, such as the light emitted by them to overlap, generating a uniform lighting on the exposure surface. The emission of each diode modulated in audio frequencies is of 2.6 mW.

The gripping system “mobile arm” type allows the adjustment of the distance between the laser diodes system and the exposure surface, existing in the homogenous magnetic field. The exposure surface is selected depending on the Petri bowl that must be irradiated and can be adjusted by means of the mobile arm.

The supply source with the modulation system. The supplying is executed from the 220V and 50Hz c.a. plug. The frontal panel comprises 11 switches, out of which one is central, for the device supplying, and ten represent the ten frequencies selected from the audio range. These are: 5Hz, 100Hz, 250Hz, 500Hz, 1kHz, 2,5kHz, 5kHz, 10kHz, 15kHz and 20kHz. The optical power emitted by the experimental device executed by us, is of 49.4 mW in pulsated regime. Taking into account the distance to which irradiation is performed, the surface on which homogenous lighting is achieved, the exposure time, and the average total power of the diodes within the device, the corresponding dosages can be computed.

Table 1 presents the optimum dosages calculated in case of irradiation with laser light modulated in audio frequencies, for homogeneously lighted surfaces, situated at certain distances from the lighting source. Irradiation doses for the two exposure times at which the experiments were performed, were selected: 0,52J/cm² is dose for the exposure time of 15 min., and 1,04J/cm² is dose for the exposure time of 30 min., respectively.

Table 1. Values of the irradiation dose corresponding to different distances, for two exposure times

T(min.)	d=5cm	d=10 cm	d=15cm	d=20cm	d=25cm	d=30cm	d=40cm
	Dose (J/cm²)						
15	8.32	2.08	0.93	0.52	0.33	0.23	0.17
30	16.63	4.16	1.85	1.04	0.67	0.47	0.34

The experiments comprised 2 samples:

a.) **Experimental samples** in which there were used microbial cultures of *Alternaria alternata* from the **A1** and **A100** types which were simultaneously irradiated with magnetic field and laser light modulated at optimum audio

frequencies of 10Hz, 500Hz and 10kHz in 2 variants with different exposure durations: 15 minutes and 30 minutes. Each variant presented 3 rehearsals each, maintained in the same conditions of work and culture.

b.) **Control sample** in which there were used non-irradiated microbial cultures (CS).

The experiments were performed in parallel, for each separate type, 3 rehearsals being executed, in the same work and culture conditions (continuous dark outside the irradiation period and 25-26°C) throughout 5 days of treatment. The determinations were performed daily for 5 days (**D1-D5**) starting with the III-rd day (after 72 hours) from the sowing of the cultures, when the first simultaneous treatment with irradiation with physical field was also performed. The results are schematically illustrated in the following tables and charts.

Results and discussions

On the basis of the experimental data and of the calculations made by means of Table 1, the graphical representation of the irradiation dose was obtained, depending on the exposure time and on the distance from the irradiation source to the surface of the bowl with inoculation culture, which is going to be irradiated (Fig. 1).

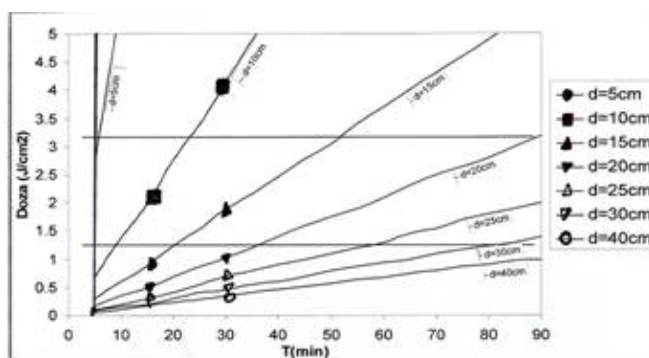


Fig 1. The irradiation dose, depending on the exposure time and on the distance «d»

The exposure surfaces increase with the increase of the distance between the irradiation source and the surface of the bowl with inoculate culture that is going to be irradiated. This leads to the decrease of the exposure doses and, implicitly, to an increase of the exposure time. Depending on the dose necessary for a given surface, the corresponding exposure time is selected.

The magnetic field used in the experiment had an intensity of 6 mT and dropped to 5 mT when the intensity of the current distributed on the network decreases.

Thus, in Table 2 and Fig. 2a there is presented the influence of the treatments produced by the simultaneous irradiation with magnetic field and field of laser light, modulated at optimum audio frequencies of 10Hz, 500Hz and 10kHz, on the cultures of *Alternaria alternata* A1 type, applied for 15 min/day throughout 5 days, in comparison to the untreated control sample. Following the performing of the daily measurements (diameter of colonies) in case of the S₁₅-10Hz experimental sample in the microbial cultures of *Alternaria alternata* A1 type, simultaneously irradiated with a magnetic field of intensity 6 mT and laser light field, modulated at audio frequencies of 10Hz for 15 min/day, it was noticed that the values obtained during the 5 days of irradiating were situated below the values registered by the untreated colonies (CS). The inhibition differences in the development of the colonies of *Alternaria alternata* A1 type recorded during the 5 days were of maximum 12.10% on irradiation day II and minimum 4.38% on irradiation day IV, compared to the average values of increase in diameter (cm) measured in the 3 rehearsals in the control sample. In exchange, when applying the treatment with laser light field, modulated at audio frequencies of 500Hz and 10kHz for 15 min/days it was noticed that the values obtained during the 5 irradiation days (Table 2 and Fig. 2a) were situated above the values recorded in the untreated colonies (CS).

In Table 3 and Fig. 2b we can see the influence of the treatments produced by the simultaneous irradiation with magnetic field and laser light field, modulated at audio frequencies of 10Hz, 500Hz, and 10kHz, on the cultures of *Alternaria alternata* A100 type, applied for 15 min/day throughout the 5 days, compared to the untreated control sample.

Table 2. Influence of treatments with 15 minutes duration of irradiation on the cultures of *Alternaria alternata* A1 type, compared to the control sample (3 rehearsals/sample)

Samples	Average values recorded when measuring ϕ cultures (cm)				
	<i>Alternaria alternata</i> A1 type				
	Irradiation day I	Irradiation day II	Irradiation day III	Irradiation day IV	Irradiation day V
CS	3,06	3,80	4,59	5,02	5,41
S15-10Hz	2,77	3,34	4,11	4,80	5,03
S15-500Hz	2,94	3,82	4,64	5,19	5,63
S15-10kHz	3,04	3,95	4,85	5,42	6,04

Where:

CS = **Control sample** = non-irradiated microbial cultures; **S15-10Hz**; **S15-500Hz**; **S15-10kHz** = microbial cultures of *Alternaria alternata* of A1 types simultaneously irradiated with magnetic field and laser light field, modulated at audio frequencies of 10Hz, 500Hz and 10kHz for 15 minutes/day.

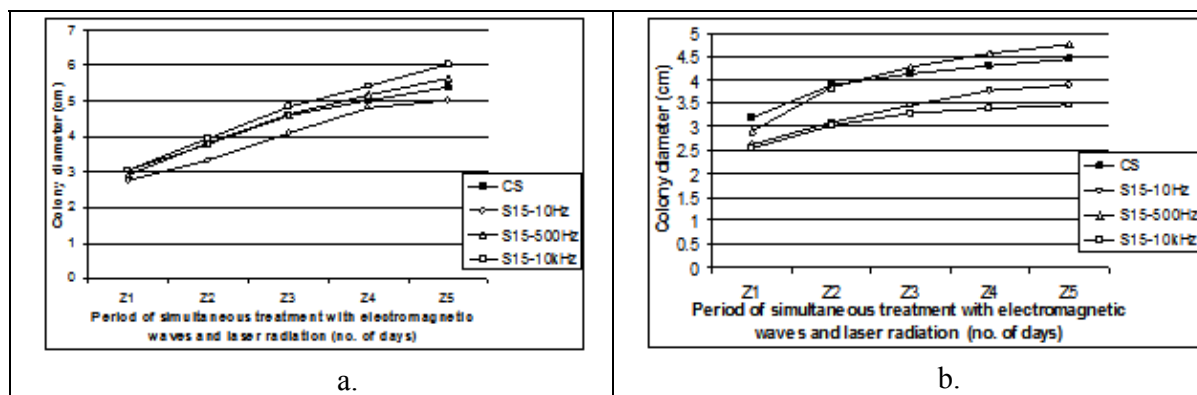


Fig 2. Influence of physical treatments with 15 minutes duration applied for 5 days on colonies of *Alternaria alternata* A1 (a) type and A100 (b) type, compared to the untreated control sample

In case of the microbial cultures of *Alternaria alternata* **A100** types it was established that during the 5 days of simultaneous irradiation with red light, at the frequency of 10 Hz and **10kHz** for 15 min/day and magnetic field of intensity 6mT, appear inhibition differences of the development of the colonies by means of the average values of increase in diameter (cm) measured at the 3 rehearsals, compared to the control sample. The results of the average values recorded in case of the experimental variant **S15-10kHz** were smaller, compared to the untreated control, with up to 22.56-22.02% (D2 with the difference of 0.88cm and, respectively, D5 with the difference of 0.98cm compared to the control) in comparison to the **S15-500Hz** experimental variant, where the inhibition of the colonies occurred in a percentage of maximum 20.25% (D2, with the difference of 0.79cm) in comparison to the untreated control (Table 3 and Figure 2b).

In the following, in Table 4 and Fig.3a there are presented the results obtained in case of applying the treatments produced by the simultaneous irradiation with magnetic field and laser light field modulated at audio frequencies for 30 min/day for 5 days, on the cultures of *Alternaria alternata* **A1** type, compared to the untreated control sample.

Table 3. Influence of treatments with 15 minutes duration of irradiation on the cultures of *Alternaria alternata* A100 type, compared to the control sample (3 rehearsals/sample)

Samples	Average values recorded when measuring ϕ cultures (cm)				
	<i>Alternaria alternata</i> A100 type				
	Irradiation day I	Irradiation day II	Irradiation day III	Irradiation day IV	Irradiation day V
CS	3,19	3,90	4,13	4,30	4,45
S15-10Hz	2,63	3,11	3,46	3,77	3,90
S15-500Hz	2,92	3,84	4,28	4,56	4,75
S15-10kHz	2,55	3,02	3,30	3,38	3,47

Where:

CS = **Control sample** = non-irradiated microbial cultures; **S15-10Hz**; **S15-500Hz**; **S15-10kHz** = microbial cultures of *Alternaria alternata* of A100 types simultaneously irradiated with magnetic field and laser light field, modulated at audio frequencies of 10Hz, 500Hz

and 10kHz for 15 minutes/day.

The efficiency of irradiations with 30 minutes duration was expressed in case of *Alternaria alternata* A1 type, by inhibiting the increase of colonies at the application of simultaneous treatments with magnetic field and laser light field modulated at audio frequencies of 10Hz, 500Hz and 10kHz compared to the non-irradiated control sample. The results of the average values not recorded in case of the S30-10kHz experimental variant were smaller in comparison to the untreated control, by 25.27% (day 3 with a difference of 1.16cm), 23,7% (day 4 with a difference of 1.19cm) and 24.02% (day 5 with a difference of 1.30cm), (Table 4 and Fig 3a.).

Table 4. Influence of treatments with 30 minutes duration of irradiation on the cultures of *Alternaria alternata* A1 type, compared to the control sample (3 rehearsals/sample)

Variant	Average values recorded when measuring ϕ cultures (cm)				
	<i>Alternaria alternata</i> A1 type				
	Irradiation day I	Irradiation day II	Irradiation day III	Irradiation day IV	Irradiation day V
CS	3,06	3,80	4,59	5,02	5,41
S30-10Hz	2,96	3,70	4,08	4,73	4,90
S30-500Hz	2,99	3,79	4,36	4,87	5,12
S30-10kHz	2,84	3,14	3,43	3,83	4,11

Where:

CS = **Control sample** = non-irradiated microbial cultures; S30-10Hz; S30-500Hz; S30-10kHz = microbial cultures of *Alternaria alternata* of A1 types simultaneously irradiated with magnetic field and laser light field, modulated at audio frequencies of 10Hz, 500Hz and 10kHz for 30 minutes/day.

In Table 5 and Fig. 3b there is presented the influence of treatments produced by the simultaneous irradiation with magnetic field and laser light field modulated at audio frequencies of 10Hz, 500Hz and 10kHz, on cultures of *Alternaria alternata* A100 type, applied for 30 min/day for 5 days, in comparison to the untreated control sample.

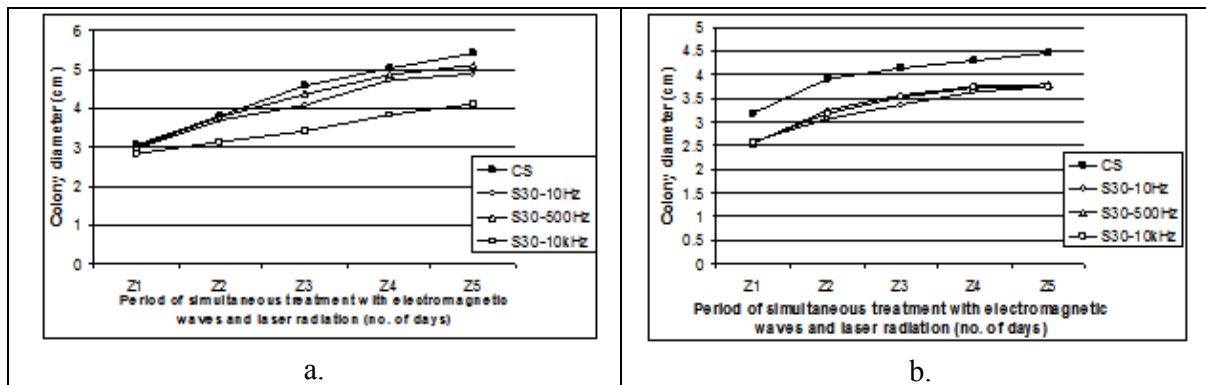


Fig 3. Influence of physical treatments with 30 minutes duration applied for 5 days on colonies of *Alternaria alternata* A1 (a) type and A100 (b) type, compared to the untreated control sample

Table 5. Influence of treatments with 30 minutes duration of irradiation on the cultures of *Alternaria alternata* A100 type, compared to the control sample (3 rehearsals/sample)

Samples	Average values recorded when measuring ϕ cultures (cm)				
	<i>Alternaria alternata</i> A100 type				
	Irradiation day I	Irradiation day II	Irradiation day III	Irradiation day IV	Irradiation day V
CS	3,19	3,90	4,13	4,30	4,45
S30-10Hz	2,58	3,06	3,36	3,63	3,76
S30-500Hz	2,54	3,24	3,56	3,75	3,80
S30-10kHz	2,57	3,19	3,52	3,72	3,76

Where:

CS = **Control sample** = non-irradiated microbial cultures; **S30-10Hz**; **S30-500Hz**; **S30-10kHz** = microbial cultures of *Alternaria alternata* of A1 types simultaneously irradiated with magnetic field and laser light field, modulated at audio frequencies of 10Hz, 500Hz and 10kHz for 30 minutes/day.

The efficiency of irradiations with an exposure duration of 30 de minutes was also proven in case of colonies of *Alternaria alternata* **A100** types, by inhibiting their growth under the application of treatments with magnetic field and laser light field modulated at audio frequencies of 10Hz, 500Hz and 10kHz, in comparison to the untreated control sample. These values were smaller starting with the II-nd day of applying the treatment (Table 5 and Figure 3b), especially at the S₃₀-10Hz experimental variant (21.53%, and, respectively, with a difference of 0.84cm) and at the la P₃₀-10kHz experimental variant (18.20%, and, respectively, with a difference of 0.71cm), in comparison to the non-irradiated control sample. In the following treatment days, the inhibition differences with respect to the control in developing the colonies of *Alternaria alternata* **A100** types in treatments with **10Hz** and **10kHz** were at close values, recording a of 15.50% difference with respect to the control on treatment day V.

Conclusions

- The inhibition of development of the **A1** type of *Alternaria alternata* was achieved in the simultaneous treatment with magnetic field and laser light field modulated at audio frequencies of **10Hz** and exposure duration of **15 minutes**, 12.10% below the control values on irradiation day II, as well as at audio frequencies of **10kHz** and exposure duration of **30 minutes**, where the recorded values were lower, in comparison to the untreated control, with maximum 25.27%.
- In case of the **A100** type of *Alternaria alternata*, the inhibition of colonies development was achieved at the simultaneous treatment with magnetic field and laser light field modulated at audio frequencies of **10kHz** and exposure duration of **15 minutes**, with 22.56% below the control values, and at audio frequencies of **10Hz** and exposure duration of **30 minutes**, the values recorded were lower that the untreated control, with 21.53%; these values were recorded, in both cases, on irradiation day II.
- However, throughout the 5 days of irradiation with red light at the frequency of **500Hz** simultaneously with the application of the magnetic field of intensity 6 mT and exposure duration of 15 or 30 de minutes, there were not established significant inhibition differences on the development of the colonies on the two types, **A1** and **A100**, of *Alternaria alternata*, compared to the control sample.

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