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PRELIMINARY RESULTS REGARDING THE TESTING OF TREATMENTS WITH LIGHT-EMITTING DIODE (LED) ON THE SEED GERMINATION OF *ARTEMISIA DRACUNCULUS* L.

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Abstract

Regardless of the food diet (raw, vegan, vegetarian, carnivore), consumption of sprouted grains of different plants, especially vegetables, is encouraged, due to their therapeutic, regenerative and alimentary benefits. Seeds and sprouts have in their composition 20 - 30 times higher amounts of enzymes, minerals and nutrients than mature plants. Artemisia dracunculus L. *(tarragon) is a medicinal and aromatic perennial herb which belongs to the Asteraceae family. It was chosen for the experiment due to its benefits, the essential oil of tarragon displaying antibacterial, antioxidant, antihyperglicemic activity. The plant is also known for enhancing digestion and having a pleasant spicy aroma.*

Studies show that light-emitting diode (LED), light exposure improves the quality of the growth, metabolism and accumulation of bioactive substances. As a result, the effect of different LED light colors on Artemisia dracunculus L. *(tarragon), seed germination was studied. The Artemisia dracunculus* L. *(tarragon), seed were exposed to white, red, blue or green LED light, over a photoperiod of 16 hours, for seven days. The red LED exposure determined a higher degree of germination and longer hypocotil height. The blue LED exposure determined a better development of cotyledons.*

The experimental results obtained contribute with useful information in order to establish a method of easily growing fresh sprouts of Artemisia dracunculus L. *(tarragon), for therapeutic and culinary use, under exposure to a low-carbon, power saving and inexpensive lightning means.*

Key words: *Artemisia dracunculus* L., *LED, seed germination.*

INTRODUCTION

Artemisia dracunculus L. is a perennial herb belonging to the *Asteraceae* family, native to Europe, southern Russia in the area of the Caspian Sea, China and western and central North America. Stems are ascending and richly branched. The plant displays lanceolate, alternate, entire leaves on a plant 45-75 centimeters high (Hemphill and Hemphill, 1984). Under certain conditions, the plant bears small, greenish-white, sterile flowers in terminal panicles. Under typical north temperate zone conditions these are seldom seen. The root is thin, branched and deep up to 30 - 40 cm. Germinative capacity lasts 2 - 3 years. *Artemisia dracunculus* L. is extremely popular as a culinary herb due to its licorice or anise flavour, which adds a special, refreshing taste to sauces and main dishes (Fox, 1970). The leaves and young stems are used for flavoring various dishes and pickles. In the food industry it is used for seasoning of canned meat and vegetables in different marinades, cucumbers and pickles in vinegar (Hassanzadeh et al., 2016). Aerial parts of the plant have traditional therapeutic use in human medicine. The active principles are eupeptic, ensuring normal digestion (Kalantari et al., 2013). Empirical medicine assigns diuretic properties to this plant. The plant is recommended in treating liver and kidney diseases, ascites, appetite and digestion stimulation, in rheumatism, headaches, toothaches (Ribnicky et al., 2004).

French tarragon does not produce viable seed, while Russian tarragon does (Fox, 1970). Consequently, purchased tarragon seed will, of necessity, be of the Russian variety, which is a taller (to 150 centimeters), coarser, weedy plant. The result of this development of a non-seeding plant is that French tarragon must be reproduced vegetative, without seeds (Hartmann and Kester, 1983). Vegetative propagation differs from seed propagation in that vegetative plant parts, such as stems, roots, bulbs, and leaves are used, rather than seed (Adriance and Brison, 1955). Traditionally, this has been done by dividing existing plants. The process is inexpensive, rapid, simple, and does not require specialized techniques like grafting or budding (Hartmann and Kester, 1983). Due to the necessity of asexual reproduction, French tarragon remains a premium-priced herb plant. The strong and aromatic smell of some species of *Artemisia* genus is due mainly to high concentrations of volatile terpenes, constituents of their essential oils, especially in leaves and flowers. The chemical composition of essential oils from the *Artemisia* genus has been extensively studied in several species from around the world. (Balza and Towers, 1984; Vienne et al., 1989; Venskutonis et al., 1996). Many studies have shown that *Artemisia* species display significant intraspecific variations in the terpene constituents of their essential oils. In some cases, the variation in the volatile components of these plants may occur during plant ontogeny or growth at different altitudes. The quality and yield of essential oils from *Artemisia* species is influenced by the harvesting season, fertilizer and pH of soils, the choice and stage of drying conditions, the geographic location, chemotype or subspecies, choice of plant part or genotype, or extraction method (Abad et al., 2012).

Variants of *Artemisia dracunculus* L. possess anti-diabetic compounds (Eisenman at al., 2014). There are studies that prove the antyhyperglicemian effect of *Artemisia dracunculus* L. The botanical extract of *Artemisia dracunculus* L. improves insulin action (Obanda et al., 2014). In experiments carried on mice, *Artemisia dracunculus* L. polyphenols complexed to soy protein displayed enhanced bioavailability and hypoglycemic activity on the subjects (Ribnicky et al., 2004; Ribnicky et al., 2006).

There are many studies that present the phytochemical benefits of growing plants under LED exposure. For example, in a study on the antioxidant activity of pea (*Pisum sativum* L.) seedlings, the contribution of red light to significant β-carotene expression and antioxidant activity for nutrition and health benefits and blue light to seedling weight and chlorophyll induction of radiated pea (*Pisum sativum* L.), seedlings are emphasized (Wu et al., 2007). Studies on algae show that the biomass and fatty acid production was improved under LED light stress (Choi et al., 2015; Zhao et al., 2013). LED lights were found to increase bioactive substances at low energy costs in culturing fruiting bodies of *Cordyceps militaris* (Yi et al., 2014). LED treatment's enhancing effects were also studied on basil, *Ocimum basilicum* (Bantisa et al., 2016), leaf lettuce, *Lactuca sativa*, (Chang and Chang, 2014, Chen et al., 2014).

LED treatment was also used, along with magnetic field and laser light field, modulated at audio frequencies, on colonies of *Alternaria alternata*, for the purpose of increasing the inactivation and inhibition percentage over these fungi (Niculiță et al., 2008; Dănăilă-Guidea et al., 2008; Ristici et al., 2008). Light irradiation with laser diodes was used also in experiments on vegetables, such as tomato seeds, *Solanum lycopersicum* L., (Niculiţă et al., 2006) or on seedlings of some annual ornamental species (Dănăilă-Guidea et al., 2011). Results show that the germination rate increases for irradiated seeds. In our experimental researches, we chose to use light-emitting diode (LED), exposure to determine seed germination of *Artemisia dracunculus* L. (tarragon).

MATERIALS AND METHODS

For the experiment there were used seeds of *Artemisia dracunculus* L. (tarragon), obtained by conventional horticultural methods. The seeds were in their dormant phase and procured from commercial producer.

Microbial contamination is a major issue for the *in vitro* culture, which causes most of the germ-plasm losses. Aseptizing the surface of the seeds of *Artemisia dracunculus* L. (tarragon) had the following stages (Badea and Săndulescu, 2001; Cachiţă – Cosma et al., 2004):

- immersion of the seeds in a solution of ethylic alcohol ($C_6H_{12}O_6$) 70% and keeping it for 1 minutes;
- successive washing of the seeds, three times for 10 minutes, in aseptized distilled water, in order to remove traces of the aseptizing agent

which, if not removed, would be harmful to the development of the inoculums.

After the process of aseptisation, the seeds of *Artemisia dracunculus* L. (tarragon), were placed in sterile, covered, transparent plastic recipients, on sterile gauze, procured from pharmacy. A number of 10 seeds of *Artemisia dracunculus* L. (tarragon), was placed in each recipient.

The necessary nutrients for the seed germination and plant development were provided by a solution of *Vitaflora Universal* procured from commerce. *Vitaflora Universal* contains the following nutrients: N 7.000%; P_2O_5 3.000%; P 1.300%; K_2O 5.000%; K 4.200%; B 0.014%; Cu 0.007%; Fe 0.020%; Mn 0.010%; Mo 0.001%; Zn 0.007%; Mg 0.010%.

For the first day of inoculation, a dilution of 1/10 of *Vitaflora Universal* solution was used. For the rest of the days of the experiment, a dilution of 1/20 was used. The nutritive solution was diluted in aseptized distilled water. Five milliliters of solution were poured over the seeds every two days.

The LED bulbs were also procured and installled on different lamps, each correspondding to a recipient inoculated with *Artemisia dracunculus* L. seeds. The recipients were placed at 15 cm distance from the LED bulb. The LED bulbs procured had the specifications from Table 1.

Table 1. The LED bulb's specifications

Technical specifications \ Light colors	Warm white	Red	Blue	Green
Power (W)	5	5	5	5
Tension (V)	220	220	220	220
Wavelength (nm)	470-640	640	470	525
Color temperature (K)	3000	3000	3000	3000
Light flux (lm)	200	200	200	200
Incandescent bulb equivalent (W)	20	20	20	20
Fascicle angle (º)	120	120	120	120
Dimensions (mm)	80*40	80*45	80*45	80*45
Life span (h)	15000	15000	15000	15000
Screw base	E27	E27	E27	E27

The seeds of *Artemisia dracunculus* L. (tarragon), were incubated at 23°C ± 2°C, with a photoperiod of 16 h and a temperature of 20°C ± 2°C during the dark period of 8 h.

The biomass of *Artemisia dracunculus* L. was dried in oven for 16 h at 105°C (Windham et al., 1987).

The experiments were repeated 2 times. Each experimental variant consisted in three repetitions. The measurements were done for each individual inoculate.

For statistical procedures, average value and standard deviation were determined for the analyzed parameter.

RESULTS AND DISCUSSIONS

The analysis methods of the studied biological material consisted of quantitative elements. As a result, the monitoring methods in order to carry out the testing of treatments with light-emitting diode (LED), on the seed germination of *Artemisia dracunculus* L. involved both, the morphometric measurements of the number of germinated seeds, hypocotyls and cotyledons, and the weight measurements of the fresh weight and dry weight sprouts. The white LED treatment was used as control experimental variant.

The percentage of germinated seeds under red LED treatment had a medium value of 90% ± 5%, higher than the control experimental variant, treated with white LED, which had a medium percentage of 80% ± 5% germinated seeds. The blue LED treatment led to a medium percentage of 50% ± 5% germinated seeds, while the green LED treatment led to a medium percentage of 40% ± 5% germinated seeds of *Artemisia dracunculus* L. (Figure 1).

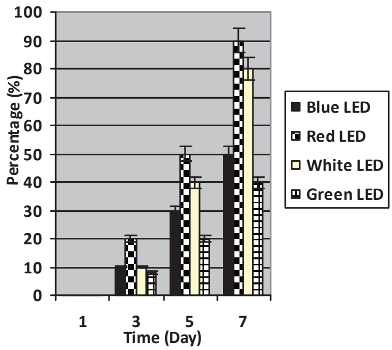

Figure 1. Medium values of the percentage of germinated seeds of *Artemisia dracunculus* L. under the influence of light-emitting diode (LED) treatments

The hypocotyl length of the *Artemisia dracunculus* L. seedlings under blue LED treatment had a medium value of 6 ± 0.34 cm, higher than the red LED treatment variant, which had a medium percentage of 5 ± 0.34 cm. The white LED and green LED treatment led to a medium hypocotyl lenght of 4 ± 0.34 cm (Figure 2).

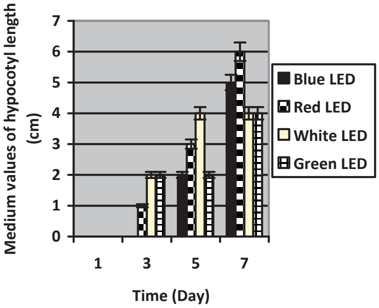

Figure 2. Medium values of the hypocotyl length at *Artemisia dracunculus* L., obtained under the influence of light-emitting diode (LED) treatments

The cotyledon length of the *Artemisia dracunculus* L. seedlings under blue LED treatment had a medium value of 0.6 ± 0.03 cm, higher than the red, green and white LED treatment variants, which had a medium percentage of 0.5 ± 0.03 cm (Figure 3).

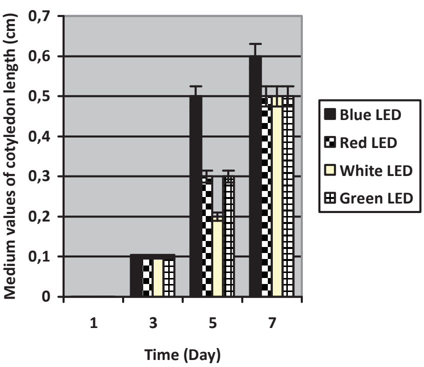

Figure 3. Medium values of the cotyledon length at *Artemisia dracunculus* L., obtained under the influence of light-emitting diode (LED) treatments

Traditional light technologies like high-pressure sodium, metal halide or fluorescent lights produce distinct light spectrum and wavelengths that are effective, but not necessarily optimized for plant growth. LEDs, on the other hand, have the flexibility to deliver specific wavelength combinations and lighting strategies that may yield faster and more favorable results for plant growers and researchers (Choi et. al, 2015, Yi et. al, 2014). LEDs retain their spectral profiles when dimmed, providing consistency in spectrum as researchers adjust intensities throughout the light cycle. Unlike fluorescent bulbs, LED output remains vastly more stable as chamber temperature decreases, providing consistency in spectrum for a wide range of temperatures (Chen, 2014). The experimental results obtained, by the testing of treatments with light-emitting diode (LED) on the seed germination of *Artemisia dracunculus* L., are in agreement with the previous studies, which state that LED treatments induce greater biomass production (Figure 4).

Figure 4. Sprouted of *Artemisia dracunculus* L. seeds on sterile gauze

The influence of the LED treatments over the *Artemisia dracunculus* L. biomass quantity was determined through the quantitative weighing of the fresh vegetal material (fresh weight) and of the dry vegetal material (dry weight). The red LED treatment determined a higher medium quantity of biomass than the blue, green and white LED treatments, represented by a fresh medium weight of 0.0490 ± 0.0020 g and a dry medium weight of 0.0028 ± 0.0002 g (Figure 5).

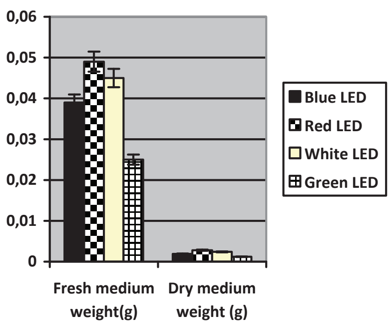

Figure 5. Fresh weight and dry weight of the *Artemisia dracunculus* L. sprouts, obtained under light-emitting diode (LED) treatment

The red LED treatment generated the best results in seed germination (90% of germinated seeds in the last day of the testing), and in hypocotyl length (the longest seedlings had a hypocotyl of 6 cm in the last day of the testing). The blue LED treatment exposure determined a better cotyledon development, cotyledons reaching 0.5 cm in length in the last days of the testing.

The green LED treatment determined poor results in both seed germination and sprouts development.

In our study, we chose to use light-emitting diode (LED), exposure to determine seed germination, due to numerous advantages (higher percentage of germinated seeds, a faster growth, longer hypocotyl size), which were proved during the experiment.

CONCLUSIONS

The monitoring of experimental research of preliminary results regarding the testing of treatments with light-emitting diode (LED), on the seed germination of *Artemisia dracunculus* L., allowed us to obtain, experimental results that indicate the conclusions:

- red LED treatment generated the best experimental results (90% percentage of germinated seeds). The red LED exposure determined a higher degree of germination and longer hypocotil height. The blue LED exposure determined a better development of cotyledons.
- the variety of *Artemisia dracunculus* L. (tarragon) displays great potential for *in vitro* studies, for therapeutical and culinary development;
- the percentage of germinated seeds of *Artemisia dracunculus* L. was higher in the case of the experimental variant which used red LED treatment;

Therefore, we studied a method of growing sprouted fresh tarragon (*Artemisia dracunculus* L.), all over the year, and obtained results for seed germination under LED exposure. The experimental results obtained contribute with useful information in order to establish a method of easily growing fresh sprouts of *Artemisia dracunculus* L. (tarragon), for therapeutic and culinary use, under exposure to a low-carbon, power saving and inexpensive lightning means.

The preliminary experimental results contribute to the completion of the necessary *in vitro* conditions for the obtaining of fresh vegetal biomass of *Artemisia dracunculus* L., both as germinated seeds and as sprouts, which can be destined to the therapeutical or alimentary consume.

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