IRON OXIDE NANOPARTICLES PHYTOTOXICITY ON *LAVANDULA ANGUSTIFOLIA*

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Abstract. Magnetic nanoparticles phytotoxicity supplying the soil culture of the *Lavandula angustifolia* plantlets (lavender) after 190 days of growth was evaluated. The lavender plantlets grown in soil and irrigated with aqueous colloidal solutions of iron oxide (Fe_3O_4) nanoparticles coated with citric acid and 11.4 nm physical diameter, having different nanoparticles concentrations ranging between 19.6 µg/mL and 686 µg/mL. The influence of the iron oxide nanoparticles presence in the culture soil of lavender plantlets was evaluated at the level of seeds germinations, seedlings length, photo-assimilatory pigments content and photosynthesis efficacy.

Key words: iron oxide nanoparticles, magnetite, phytotoxicity, chlorophylls, *Lavandula angustifolia.*

INTRODUCTION

Nanotechnology can enhance the quality of human life through its biological applications in diverse fields. In the last decade, the field of biological applications of nanoparticles experienced a high attention for magnetic nanoparticles use in treatments or diagnostics [5]. In biological applications, the magnetic nanoparticles may be biocompatible or not, thereby some questions have been raised about the potential action of these on the environment and living.

Phytotoxicity of metallic nanoparticles and their accumulation into vegetal organisms is one of the present-day research topics, considering the impact of engineered nanoparticles on the environment elements.

Being an important component of the ecosystem plants provides a potential path for nanoparticles transport to the environment, conducting to their accumulation into the food chain. There are research papers in the scientific literature that have been noticed the nanoparticles assimilation and intracellular localization at plants level and results reported concluded that each type of nanoparticles exhibits a

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preferred pathway for cellular internalization [19]. According with some researches results the nano-sized particles can enter into vegetal organisms through the leaves and/or root cells path [6]. The magnetite particles accumulation has been detected in different plants species using magnetic methods by Khalilov *et al.*, concluding that plants are able to absorb magnetic nanoparticles from the soil [9]. The plant cell wall functions as a porous network that decides the upper size limit for the external particles which could go through the cell wall. This limit is conditioned by the pore diameter of the cell wall, having a size range between 5 and 20 nm [4]. Hence, the magnetic nanoparticles with a diameter up to 20 nm, could easily cross the plant cell wall and get to the plasma membrane [7].

Also, the surface of nanoparticles could be responsible for phytotoxicity, due to the redox reactions in contact with molecules of tissues and by ions releasing with an enhanced toxicity grade from the surface of nanoparticles. Presence of the nanoparticles could cause oxidative stress at tissues level leading to the biological damages [2].

Positive influence on the plant growth due to the iron oxide nanoparticles presence in culture medium has been explained on the iron role basis in the vegetal cell metabolism [3]. Also, the positive effects of the magnetic nano-sized particles on the chlorophylls content have been revealed in some scientific researches [11–12, 14]. The present paper is focused on the investigation of the effect of iron oxide nano-sized particles added in the culture soil of *Lavandula angustifolia* plantlets on their development and photosynthesis process efficacy. These results could supply the scientific knowledge in the research field of vegetal organism reaction to iron oxide nanoparticles presence, due to the irrigation of plants soil with magnetite/citric acid nanoparticles.

MATERIALS AND METHODS

Iron oxide nanoparticles coated with citric acid ($C_6H_8O_7$) used in this experimental study were prepared by chemical precipitation of $FeCl_2$ and $FeCl_3$ stock solutions in alkali medium at room temperature method, as described in Răcuciu, 2009 [15]. By means of IR spectra analysis, has been shown in chemical composition that magnetite (Fe_3O_4) are the core of magnetic nanoparticle.

Nanoparticles with spherical shape were evidenced by transmission electronic microscopy by imaging analyses of 10^4 diluted aqueous suspensions (provided by a TESLA device with 1.0 nm resolution). According with TEM imaging analyses, the iron oxide nanoparticles coated with citric acid, used in this experimental study, had average diameter of about 11.4 nm and dimensional distribution ranging between 4 and 22 nm. Citric acid coated magnetic nanoparticles native suspension had $5 \cdot 10^{23}$ nanoparticles/mL and saturation magnetization of 23 kA/m (data recorded by Gouy method and published previously in [15]).

We can assume that iron oxide nanoparticles, stabilized with citric acid, could be biocompatible and having relevance in plants biotechnology, since citric acid is a natural product of citrus fruits, some berries or other fruits, being added to many foods as preservative and as flavoring. Also, it's known that the magnetite (Fe_3O_4) is a biocompatible compound.

It was chosen *Lavandula angustifolia* (lavender) seeds as biological material, due to its relevance for pharmaceutical and food industry. Lavender seeds were provided by Fares-Orăştie, România from an experimental lot. Germination took place on porous filter paper pad in Petri dishes (with 1.5 g amount of seeds per dish) moistened with distilled water (control case), with citric acid solution (CA variant) and, respectively, with diluted iron oxide nanoparticles (IONPs) aqueous suspension of certain concentration (IONPs treated samples), in darkness and temperature of 24±0.5 °C. After seeds sprouting 50 viable seeds were grown in standardized flower soil, "Florisol" type, 100% natural, with 60–70% humidity and pH 7. Those two experimental samples for each case (controls and IONPs treated samples) were arranged in vessels of the same type and having 150 mL volume.

Daily supply of every experimental sample with the same amount of liquid (10 mL) was supported for 190 days of plants development. Young plantlets development was managed in controlled laboratory conditions (22.0 °C temperature; illumination with 370 lx intensity for 11 h per day; humidity of about 60%) into a laboratory climate room.

The iron oxide nanoparticles aqueous suspensions with volume fractions of 10 – 50 – 100 – 150 – 250 – 350 µL/L equivalents with $Fe_3O_4/C_6H_8O_7$ concentrations about 19.6 – 98 – 196 – 294 – 490 – 686 µg/mL, was added daily to soil of experimental IONPs treated samples during their growth for 190 days. The samples supplied with increasing concentrations of nanoparticles will be further called P1–P6. The development of the controls was done in the same laboratory conditions and the soil substrate was irrigated only with distilled water. Supplementary control group was settled from plantlets grown on the soil substrate irrigated with 0.2 g/L citric acid solution (CA control case). After 190 days of growth, using spectrophotometric methods and a UV-VIS spectrophotometer (JASCO V530), the chlorophyll a, chlorophyll b and total carotenoid pigments levels (photo-assimilatory pigments) in the fresh tissues of lavender were analyzed. The evaluation of the assimilatory pigments extracts (80% acetone) was accomplished using Lichtenthaler and Welburn's method [10]. The individual length of each lavender seedling was established with 10^{-3} m precision. The green tissue amounts were quantified with an analytical balance (AS220.R2 -RADWAG, Poland) with 10^{-4} g precision.

Experimental data were worked using Microsoft Excel soft package and *Statistica v.7.0*, to evaluate reliability of IONPs induced changes. Results from two experimental samples for each case (controls and IONPs treated samples), are shown as mean value ± standard deviation. The statistical interpretation of results

was achieved using *Systat v.10* software. Descriptive statistic parameters were determined for every experimental data set. Differences with $p < 0.05$ took into account as significant and were established by using ANOVA test for means comparison. By means of Student t-test with confidence levels $p = 0.01$; 0.05; 0,001, the statistic analysis of plantlets length was accomplished. Regression analyses have been done in *Statistica v7.0* software.

RESULTS AND DISCUSSIONS

The iron oxide nanoparticles presence in germination medium has led to inhibition of seeds germination. On the twentieth day of germination process, the germination percentages were as follows: 75.7% (control), 70.4% (CA control), 73.5% (P1 – 10 µL/L), 69.5% (P2 – 50 µL/L), 65.9% (P3 – 100 µL/L), 65.1% (P4 – 150 µL/L), 64.2% (P5 – 250 µL/L) and 61.1% (P6 – 350 µL/L).

After the transfer in soil, lavender seedling growth was daily checked for 190 days. In comparison with other experimental studies at the *Zea mays* level [16], when the young plantlets have presented toxic effects at direct visual inspection, in the lavender case didn't observe such effect. Lavender young plants were healthy and vigorous during whole experiment (Fig. 1).

Fig. 1. 120 days old lavender plants – experimental samples: controls (C and CA) and samples supplied with 10, 50, 100, 150, 250 and 350 µL/L iron oxide nanoparticles (P1–P6).

In the Figure 2, the average length of the lavender seedlings for all experimental samples is presented. At the lowest iron oxide nanoparticles concentration (19.6 µg/mL) a stimulation effect on the young plants growth was observed while for higher iron oxide nanoparticles concentration (> 294 µg/mL) an inhibitory effect was recorded.

An increase of over 10% than the control was revealed in the IONPs treated probes corresponding to about 19.6 µg/mL and for higher volume fractions of the iron oxide nanoparticles solutions, the stimulatory effect diminished gradually

reaching and getting down below of the control sample level, the average length decreasing up to 13% for highest iron oxide nanoparticles concentrations. Insignificant changes for average length in CA control case (seedlings irrigated only with citric acid solution) were revealed. Because the photosynthesis process is very important in vegetal organism development, this experimental study was also focused on quantification of the chlorophylls and carotenoids level (photo-assimilatory pigments) in the fresh tissue harvested from 190 days old lavender plants, grown in different experimental conditions.

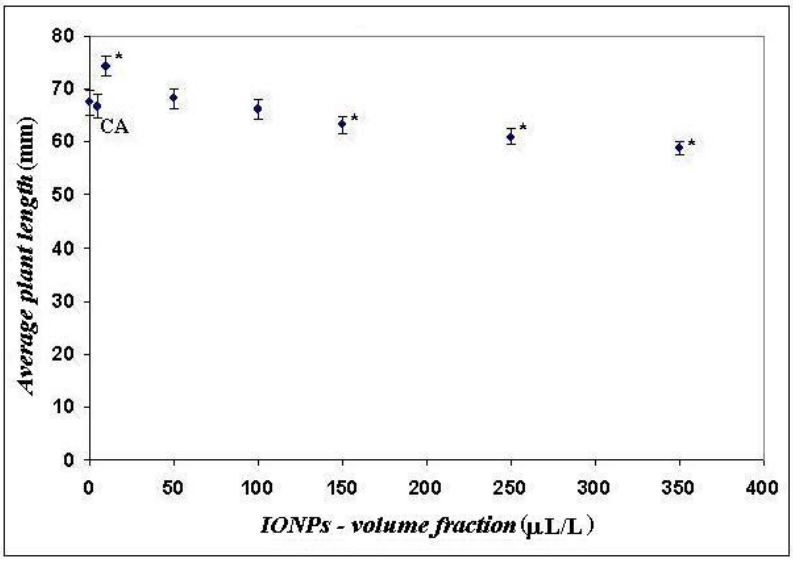

Fig. 2. The mean length of *Lavandula angustifolia* plantlets after 190 days of growth; * – statistically significant at $p = 0.05$ confidence level; CA – control samples grown in presence of citric acid solution.

As shown in Figure 3 graphical plots, the content of the chlorophyll a was got decreased for all iron oxide nanoparticles concentrations used in this experiment (up to 30%) as against to the controls ($p < 0.05$). Similar response resulted for the total carotenoids content, decreasing up to 30% for highest volume fraction of the iron oxide nanoparticles solution used in this experimental study. The chlorophyll b (*Chl b*) level was increased up to 11% for lowest iron oxide nano-sized particles concentration while for highest concentration was decreased with 28% than control one. For all analyzed assimilatory pigments, the CA control case sample revealed only non-significant variations.

Regression analysis for chlorophyll a content with iron oxide nanoparticles concentration variation using *Statistica* soft, revealed a grade 3 polynomial function with determination coefficient $R^2 = 0.978$ (Fig. 4). The total level of the

photo-assimilatory pigments was also obtained, noticing a statistically significant linear regression with increasing of the iron oxide nanoparticles concentration added in the substrate soil of lavender plants (Fig. 5).

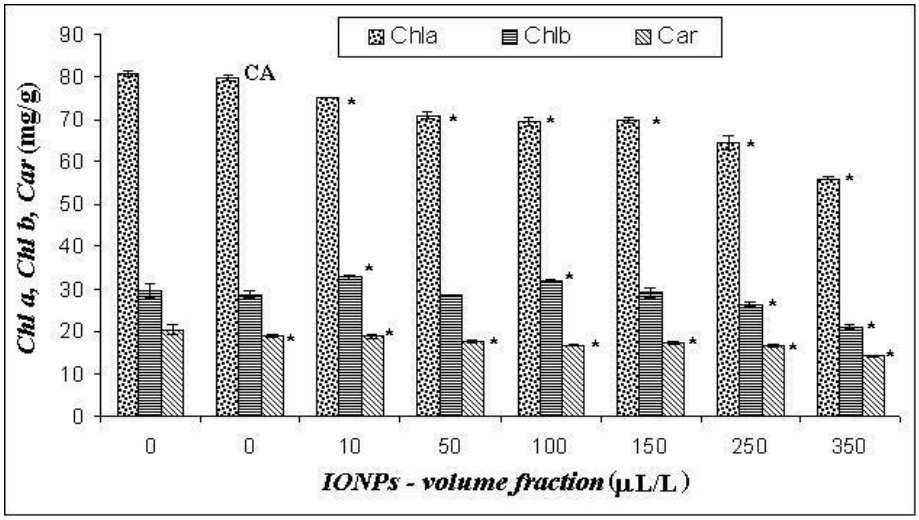

Fig. 3. Photo-assimilatory pigments level in *Lavandula angustifolia* plantlets; *Chl a* – chlorophyll a, *Chl b* – chlorophyll b, *Car* – total carotenoid pigments; * – statistically significant; CA – control samples grown in presence of citric acid solution.

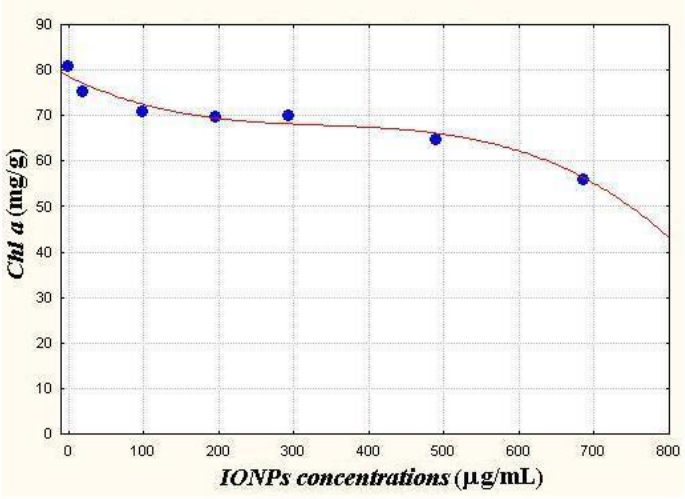

Fig. 4. Chlorophyll a levels in *Lavandula angustifolia* plantlets (*Chl a*) versus the iron oxide nanoparticles concentration added in substrate soil (µg/mL). The curve indicates the best mathematical model obtained with *Statistica* software (R^2 = 0.978).

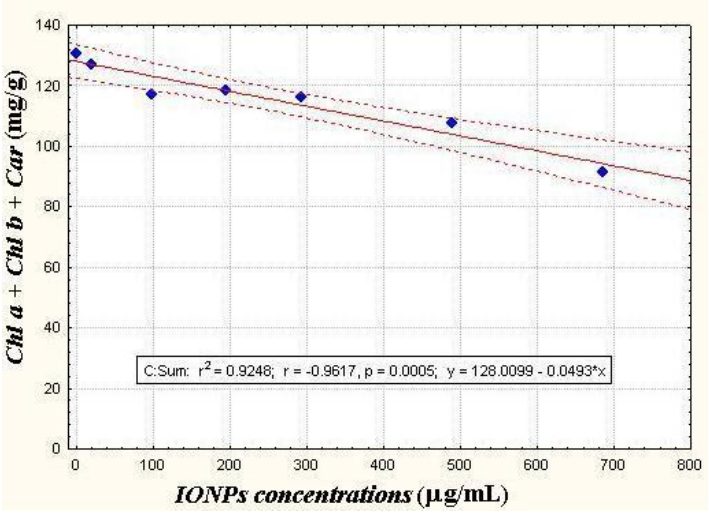

Fig. 5. The effects of iron oxide nanoparticles concentration on chlorophylls sum content (*Chl a + Chl b + Car*) (p = 0.05). The continuous line indicates the best mathematical model obtained with *Statistica* software (R^2 = 0.9248). The discontinued curves indicate the confidence interval (P = 95%).

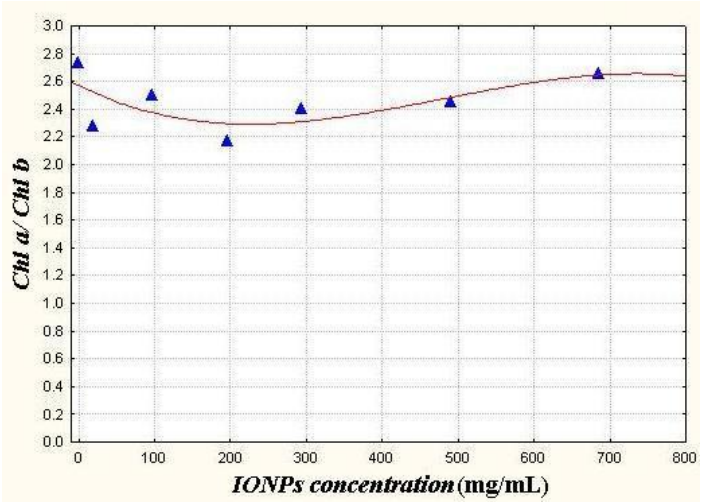

Fig. 6. The chlorophylls ratio (*Chl a/Chl b*) versus iron oxide nanoparticles concentration added in lavender plantlets soil. The line indicates the best mathematical model obtained with *Statistica* software (R^2 = 0.676).

The chlorophylls ratio (*Chl a/Chl b*) was slightly affected by the iron oxide nanoparticles presence in the soil (Fig. 6). This biochemical ratio can provide indirect information on the sensitivity of the photosynthetic system (LHC II) at the chloroplasts membranes level to the external factors. As shown into the Figure 6,

chlorophylls ratio decreased up to 20% when the iron oxide nanoparticles concentration was about 196 µg/mL ($p < 0.05$). These results could lead to the conclusion that the iron oxide nanoparticles have capacity to influence the LHC II enzyme system, which is very important in plants development. The ratio of chlorophylls sum to the all assimilatory pigments sum was not significantly changed under the iron oxide nanoparticles presence in lavender plants culture substrate (Fig. 7). The total content of chlorophyll a and b can be considered as a parameter of the total amount of light harvesting and the electrons conveyance from the chloroplast membranes [17]. Terry (1983) has considered that using iron stress could affect the light-harvesting and electrons conveyance capacity involved into photosynthesis process [17].

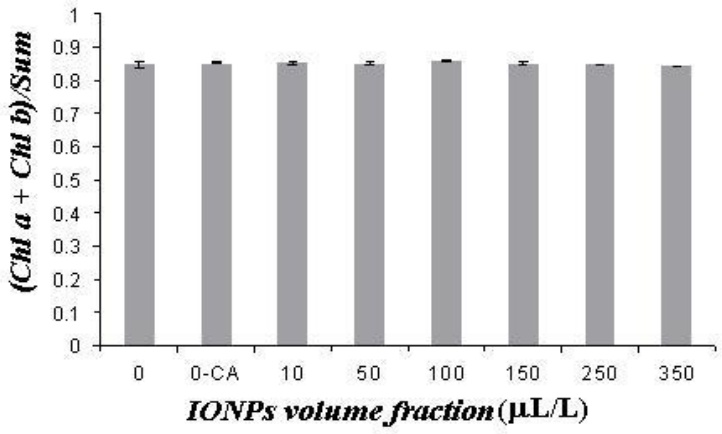

Fig. 7. The ratio of chlorophylls sum to the all assimilatory pigments sum levels in *Lavandula angustifolia* plantlets (*Chl a* – chlorophyll a, *Chl b* – chlorophyll b, *Sum = Chl a + Chl b + Car*; *Car* – total carotenoid pigments); CA – control samples grown in presence of citric acid solution.

In the attempt to explain phenomenologicaly the iron oxide nanoparticles influence on vegetal organisms it should be involved both chemical and magnetic influences, considering whole nano-sized metal oxide structure. Iron oxide nanoparticles are capable to circulate through the vascular system of plants [6], the magnetic nanoparticles being able to enter into plant tissues by means of the plasmodesmata channels [8]. These channels with mean diameter about of 50 nm have significant contribution in cellular connection. Thus, iron oxide nanoparticles with a diameter of about 11.4 nm, as the most frequent ones used in our experimental study, could enter into the membranes of the young lavender plants. The plasmodesmata channels with small diameter could stop some iron oxide nanoparticles to moving from one cell to another, by change of protein properties [13]. Thereby, iron oxide nano-sized particles with bigger size could remain blocked in the cell wall, or in near touch with the membranes. In similar cases the

magnetic characteristics of the nanoparticles could affect the ion flows occurring across cell membranes, therefore influencing the cell biochemistry including the assimilatory pigments synthesis.

Binhi has evaluated that the magnetic moment of the magnetic nanoparticles discovered in living organisms is $10^7 \div 10^9$ times superior to the value of the elementary magnetic moment noted in physics [1]. Therefore, this could conduct to considerable influences on the electric charged ions that flow through cell membranes of the vegetal tissues. The magnetic nanoparticles that entered into the plant cells by means endocytosis process could be further degraded by lysosomal digestion, releasing iron ions [18], which can be used by many cellular processes.

CONCLUSIONS

This experimental work concluded that iron oxide nanoparticles presence in the plant soil conducted to biochemical changes at *Lavandula angustifolia* plantlets during their development. The lavender seeds germination was inhibited by higher nanoparticle concentrations added in the substrate. An increase of over 10% than the control was revealed in the IONPs treated probes corresponding to about 19.6 µg/mL and for higher volume fractions of the iron oxide nanoparticles solutions, the stimulatory effect diminished gradually reaching and getting down below of the control sample level; the average length decreasing up to 13% for highest iron oxide nanoparticles concentrations. Photosynthesis efficacy, by means chlorophylls ratio, appeared to be slight inhibited by iron oxide nanoparticles presence in the soil; chlorophylls ratio decreased up to 20% when the iron oxide nanoparticles concentration was about 196 µg/mL ($p < 0.05$). The chlorophyll a and total carotenoids contents got decreased with up to 30% for higher concentrations of iron oxide nanoparticles solutions. The chlorophyll b level was increased up to 11% for lowest iron oxide nanoparticles concentration while for highest iron oxide nanoparticles concentration was decreased with 28% than control one. A negative influence of the nanoparticles presence to the lavender plants development could lead to development of lavender plants with active principles affected by.

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