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The use of organic nano-supplements of fertilizer for lily forcing period

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Abstract: *Lilium* is one of the most important ornamental plants after roses, carnations and chrysanthemum in the world that is requested as potted of cut flowers. It is so important to consider its quality along with its production rate in terms of yield (quantity). However, it always needs to intend the product costs aside from quantity and quality. Fertilizer utilization is so important and this may be improved by compounds that promote it and also have synergetic effects themselves. We examined both carboxymethylated chitosan (CMC) and magnetic nano-carbocymethylated chitosan (MNCC) to produce *Lilium* bulb and advised them especially magnetic chitosan. In this study, these compounds (magnetic and non-magnetic chitosan) at concentrations of zero, 2.5, 5, 10 and 15 mg/l were examined during lily forcing in three cultivars, including Cherbourg, Navona, Brunello, which are from Asiatic and Oriental lilies. The results showed that highest concentrations (10 and 15 mg/l) among between examined concentrations regardless of compounds types and cultivars did not make toxicity and had significant effects on plants biology and physiology [contents of carbohydrates and enzymes affecting these carbohydrates (amylases)]. However, for observing morphological changes may be need to use higher concentrations of these compounds. Note that this needs to examine the non-toxicity of higher concentrations in future studies.

1. Introduction

Flowers have always had a valuable place among different classes of society and various ceremonies and rituals. *Lilium* is one of the most important ornamental plants after roses, carnations and chrysanthemum in the world that is mostly used as cut flowers throughout the year. Furthermore, it is used as potted plants in gardens and green landscapes (Shafiee-Masouleh *et al.*, 2014). It is necessary to consider the nutrition of lilies like other plants in the greenhouse production. And also, optimization and improvement of photosynthetic efficiency of plants can be effective in increasing photosynthetic storages and thus increasing its quantitative and qualitative yields in terms of flower number, plant size and vase life. Different groups of lilies (Asiatic, Oriental and *L. longiflorum*) have different nutritional requirements. For example, Asiatic lilies has been reported that have the best growth when are fertilized by lower levels during forcing (Treder, 2003).

Chitosan particles in nano-scale can be important in the delivery of drugs in medicine, because these compounds promote the absorption of active molecules or compounds through the cell membrane and allow organs to have bioavailability to molecules. Chitosans can be used as encapsulated nanoparticles or used directly and therefore, the surface of these particles with chelating structure of the modified chitosan can play an important role in sustainable agriculture (Kashyap *et al.*, 2015). Magnetic chitosan nanoparticles with the effect of chelating elements in the experiment conducted by Shafiee-Masouleh *et al.* (2014) increased the biomass of *Lilium* and the storage organs (bulbs) during bulb production. The effect of magnetic fluids on living systems has been studied both in medicine and in the world of plants, and it has been shown that magnetic fluids can be effective in the production of calluses and metabolic activities. The effects of magnetism on plants by Earth's magnetic field, pulsed and inductive constant magnetic fields, an electromagnetic field, and effects of magnetic nanoparticles have been studied by researchers and theirs roles have been confirmed to enhance photosynthesis and growth and development of plants (Pavel *et al.*, 1999; Răcuciu and Creangă, 2007 b; Răcuciu *et al.*, 2009; Shafiee-Masouleh *et al.*, 2014). It has also been shown that superparamagnetic nanoparticles, which are as permanent magnets effected by external magnetic fields or ambient temperature of their environment, affect membrane systems and membrane ion exchange (Pavel and Creangă, 2005; Faeghi and Seyedpour, 2013). Many researchers investigated the effects of chitin and the chitosan polymers and oligomers as spraying, soil application or seed treatment and fertilizer treatments, they reported that photosynthesis and plant growth were enhanced (El-Tantawy, 2009; Dzung *et al.*, 2011; Farouk and Amany, 2012).

The allocation of carbon in plants is important that is affected by various factors such as the level of photosynthetic compounds, the number and size of competing sink (flowers, seeds, fruits, bulbs and tubers, etc.) and their location in plants and the potential for initial storage in the leaves and re-translocation in plants. Understanding the components that affect the initiation and development of organs and the balance between source and sink organs are essential to regulate the allocation of assimilates (Du Toit, 2001). The *chl* content of the leaf affects photosynthesis, and *Chl* a/b is the best index to understand the photosynthetic capacity and direct information on the activity of the enzymes involved in the photosystem II in the chloroplast membrane (Răcuciu and Creangă, 2007 b; Răcuciu *et al.*, 2009). Iron deficiency reduces the amount of photosynthetic pigments. In addition, the electron transfer in the photosystems I and II undergoes a change. Also, the activity of 1, 5-D-phosphate-carboxylase and the photosynthetic function of plant decrease. Therefore, it is necessary to provide iron for the plant, because the biochemical properties of iron and its effect on the metabolic pathway of the plant are important. Uptaking iron in alkaline soils is difficult; this is due to the formation of ferric hydroxide in the presence of oxygen, because plant cannot uptake it (Thoiron and Briat, 1999). The main component in reducing NO_3^- absorption is iron deficiency. Ferrous enzymes (nitrite and nitrate reductases) affect the NO_3^- absorption. In addition, growth of plants decreases due to decreased NO_3^- absorption and the synthesis of metabolites (proteins, nucleic acids, chlorophylls, etc.) (Borlotti *et al.*, 2012). The use of chelating agents is useful to remove iron uptake problems by plants (Abadía *et al.*, 2011). Magnetic fluids at suitable concentrations have a positive effect on the photosynthetic capacity of plant. Iron ions in the structure of magnetic fluids can be an important source of iron for the development of plants (Răcuciu and Creangă, 2007 b). Iron is not in the *chl* structure, but it is one of the essential elements to synthesize *chl* (Răcuciu *et al.*, 2009). In general, magnetic fluids are nanoparticles dispersed in water or in a hydrocarbon fluid such as citrate. Biocompatibility of magnetite (Fe_3O_4) has been confirmed (Răcuciu *et al.*, 2009). However, treating plants with electromagnetism or any other magnetic field with these particles is destructive and changes the genotype and phenotype of plants and causes chromosomal deviations (Pavel *et al.*, 1999; Pavel and Creangă, 2005; Răcuciu and Creangă, 2007 a, b; Răcuciu *et al.*, 2009).

Chitosan is one of deacetylated derivatives of chitin. As a natural polymer is abundant and can be degraded by biological agents and it can be used in agriculture. This molecule is environmentally friendly and non-toxic that is used in the formulation of slow release fertilizers (Wu and Liu, 2008). In vitro use of chitosan in a suitable concentration in *Vitis vinifera* L.

stimulated the photosynthesis and increased plant growth with increasing root and shoot biomasses. It also protected the plant against *Botrytis cinererea* fungus and cytological changes (Barka *et al.*, 2004). We utilized the effects of chitosan, as nanoparticle with magnetic properties on plant growth and development in lily bulb production (Shafiee-Masouleh *et al.*, 2014) and cucumber (the data were not published yet).

Modern agriculture should look for factors that while having positive effects as well as its use to be easy, affordable and reasonable. It is not possible to use a large magnetic field in farms. Also, use of macromolecule of chitosan as foliar application will cause stomata obstruction and reduce gas exchange and photosynthesis. Therefore, the achievement of a soluble compound or nano-structured composite with the synergic effects of two compounds (magnetite and chitosan) will be very valuable at the same time. To achieve these purposes, this research studies the following: i) Increasing yield and post-harvest quality of cut lily by increasing the efficiency of nutrient uptake and photosynthetic performance of the plant; ii) Institutionalization of chitosan use at the nanoparticle scale during forcing of lily flowers; iii) Investigating the effect of magnetic nanoparticles on yield and quality of lily flowers; and iv) Physiological and morphological understanding of the effect of chitosan nanoparticles on the yield and quality cut flowers of lily.

2. Materials and Methods

Plant material and cultivation conditions

In this study, bulbs of three cultivars of *Lilium,* including Brunello (Asiatic, 16-18 cm in circumferences), Navona (Asiatic, 16-18 cm in circumferences) and Cherbourg (Oriental, 18-20 cm in circumferences) were used to study effects of two organic supplements of fertilizer (OSFs).

After melting of vernalizing substrate of bulbs, the bulbs were disinfected with 0.1% benomyl fungicide for 15 min and dried in the air for one day. Thirty uniform bulbs in size were selected per cultivars to force. The culture substrate contained cocopeat and perlite (1:1, v/ v) that was completely homogeneous. The bulbs were planted in one-kilogram plastic bags with some pores into 10 cm depth and irrigated with tap water until emergence. After emergence every 2-3 days depending on the weather and substrate conditions were fertigated.

Greenhouse experiments in the research greenhouse of the Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran in latitude of 37° 12’ 3.94’ N and longitude of 49 38’ 55.78’ E were performed.

Preparation of fertilizer supplements

Carboxymethylation of chitosan. The carboxymethylated chitosan (CMC) was prepared from chitosan [poly (d-glucosamine), $(C_6H_{11}NO_4)_n$, deacetylated chitin] with low molecular weight 100,000-300,000 (Acros, Acros Organics, Geel, Belgium) to increase solubility of chitosan by carboxymethylation. The procedure of preparing of CMC was presented by Shafiee-Masouleh *et al.* (2014). An IR (IR-470, Shimadzu, Kyoto, Japan) (Fig. 1) was used to analyze the produced H-form CMC (not salt containing Na ion).

Preparation of nano-particles magnetite

According to Shafiee-Masouleh *et al.* (2014), nano-particles of Fe_3O_4 were synthesized. $FeCl_3$ (Merck, Germany) and $FeCl_2.4H_2O$ (Merck, Germany) were used to prepare Fe_3O_4. The whole procedure of preparing Fe_3O_4 can be observed at the mentioned paper.

Preparation of magnetic nano-carboxymethylated chitosan

The magnetic nano-carboxymethylated chitosan (MNCC) was prepared with encapsulation of Fe_3O_4 by carboxymethylated chitosan (CMC) according to

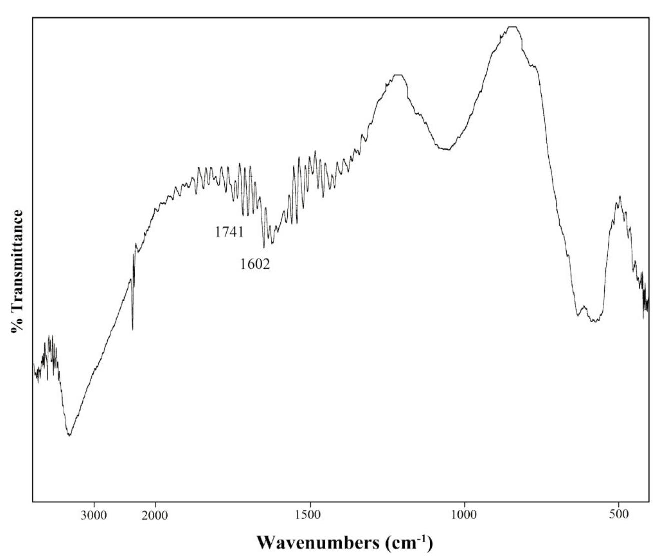

Fig. 1 - IR spectrum of carboxymethylated chitosan. This spectrum shows that the carboxylmethyl groups (-CH3COOH; 1741 cm-1; stretching vibration feature of C=O groups) resulted from monochlroacetic acid salt are linked to O atom in -OH group, and $-NH_2$ exists on carboxymethylated chitosan.

Shafiee-Masouleh *et al.* (2014). This was carried out by carbodimiide (Merck, Germany) and saline phosphate buffer in sonication conditions. The details of laboratory procedures can be studied in the mentioned paper. Analyses of the size and morphology of nano-particles were performed with SEM (Fig. 2), and IR and XRD were used to identify the coating and structure of MNCC (Figs. 3 and 4).

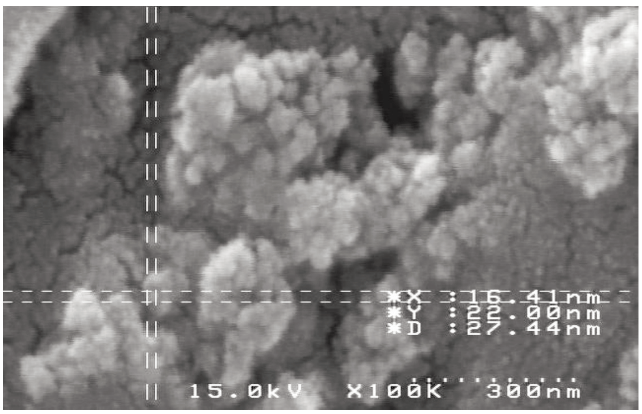

Fig. 2 - The image of magnetic nano-carboxymethylated chitosan particles by SEM microscopy. Size and morphology of Fe_3O_4 coated by carboxmethylated chitosan.

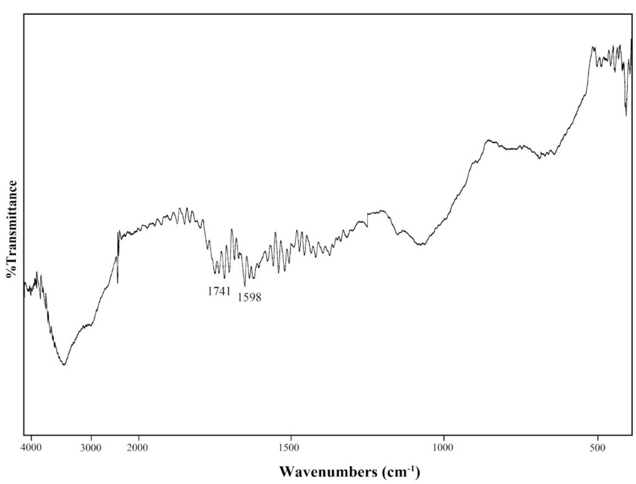

Fig. 3 - IR spectrum of magnetic nano-carboxymethylated chitosan. Transferring the absorbance band of -NH2 group from 1589 cm^{-1} to 1602 cm^{-1} and increasing absorbance intensity in wavelength number 1741 cm^{-1} show ester bond into some parts of carboxy groups in carboxymethyl chitosan on the surface of Fe_3O_4 nanoparticles.

Statistical design

A split plot factorial experiment was conducted in a completely randomized design with 15 main plots and 9 (1 + 8) sub plots. The main factors included three *Lilium* cultivars and sub factors were two types of compounds as organic supplements of fertilizer (OSFs); including carboxymethylated chitosan (CMC)

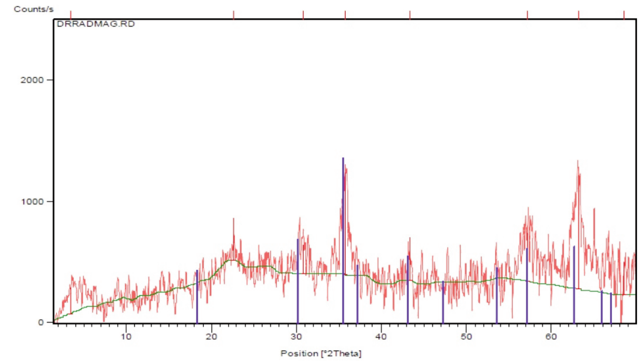

Fig. 4 - XRD pattern of magnetic nano-carboxymethylated chitosan. The widths of large peaks report crystal properties of magnetic nanoparticles. The average of particles sizes based on Debye Scherrer equation was calculated 10 nm.

and magnetic nano-carboxymethylated chitosan (MNCC) both of them at concentrations of 0, 2.5, 5, 10 and 15 mg/l. One group of zero concentration was considered as control treatment for both of compounds. The experiments were carried out by 5 replications and with 135 pots in the research greenhouse of the Faculty of Agricultural Sciences, University of Guilan, Iran. At the end of forcing period, the growth and morphological characteristics were measured for each plant (in puffy bud stage: when first flower bud showed the color and little opening in top of bud) and were harvested at a height of 45 cm from the final flower bud and transferred to the laboratory. After the stems were cut diagonally under water, they were placed into a 1000 ml bottles containing 500 ml distilled water and transferred to the growth chamber to study the post-harvest life and biochemical properties.

Variance analysis of data was measured using SAS software (version 9.1, 2003). Mean comparisons were performed with Tukey's test at probability levels of 5 or 1% according to variance analysis.

3. Results and Discussion

Magnetic nanoparticles (Pavel *et al.*, 1999; Pavel and Creangă, 2005; Răcuciu and Creangă, 2007 a, b; Răcuciu *et al.*, 2009) in maize and barley, constant magnetic field (Dhawi *et al.*, 2009) in a palm tree, pulsed magnetic field (Radhakrishnan and Kumari, 2012) in soybeen, chitosan (Górnik *et al.*, 2008) in grape and chitosan oligosaccharides (Dzung *et al.*, 2011) in coffee seedlings have been investigated. All of researchers reported an increase in mineral uptaking by the effects of magnetism or chitosan, separately.

Based on the results of 3-way ANOVA test (Table 1), effects of OSFs on vegetative development of three lily cultivars due to the uniform variances of data around the mean and Type I Error did not show a significant differences for 3-way interactions between cultivars, compounds and their concentrations. Only a significant 2-way interaction (between cultivar and concentrations of OSFs) was observed on the *chl* intensity by ANOVA test. And also, it was observed significant differences between cultivars (Table 1).

Unlike variance analysis, significant differences between three factors were observed on the vegetative length, total length and *chl* intensity. Differences between treatments on vegetative development showed that morphological characteristics are less affected by the experimented OSFs and their concentrations or these compounds may have decreasing effects on these characteristics. In Cherbourg cultivar, the highest length of vegetative stem was observed in the absence of OSFs or at low concentration (5 mg/l) of CMC, which showed a significant difference with application of 2.5 mg/l CMC in Navona. Of course, these differences were not significant com-

Table 1 - Effects of OSFs on vegetative development of three lily cultivars

Cultivars (A)	Treatments		Vegetative length (cm)	Total length (cm) [W]	Chl intensity (SPAD index)
	OSFs (B)	Cons of OSFs (mg/l) (C)			
Cherbourg	CMC	0	56.08±2.39 a	84.16±2.51 a	46.62±1.76 efg
		2.5	54.48±5.14 ab	81.36±5.40 ab	48.52±2.43 defg
		5	57.70±2.14 a	84.60±3.52 a	42.46±2.74 ef
		10	52.30±3.31 ab	79.80±3.98 ab	42.16±2.22 ef
		15	54.10±1.49 ab	81.40±3.34 ab	46.70±2.35 efg
	MNCC	0	56.08±2.39 a	84.16±2.51 a	46.62±1.76 efg
		2.5	55.30±2.91 ab	82.00±3.29 ab	46.50±3.21 efg
		5	53.00±3.41 ab	81.40±4.41 ab	46.32±1.99 efg
		10	53.12±1.36 ab	80.60±1.93 ab	39.98±2.83 g
		15	50.84±2.73 ab	82.20±2.65 ab	46.02±1.99 efg
Navona	CMC	0	48.30±2.23 ab	81.20±3.01 ab	59.52±0.31 abcd
		2.5	47.60±2.01 b	78.00±1.92 ab	60.16±1.54 abcd
		5	50.80±2.00 ab	81.80±1.57 ab	61.60±0.58 abc
		10	50.30±1.51 ab	81.90±2.65 ab	61.98±1.15 ab
		15	46.20±1.83 ab	76.00±2.07 ab	61.40±1.57 abc
	MNCC	0	48.30±2.23 ab	81.20±3.02 ab	59.52±0.31 abcd
		2.5	39.60±0.80 ab	69.90±0.48 ab	57.10±3.82 abcde
		5	49.30±2.22 ab	80.00±1.25 ab	60.16±1.13 abcd
		10	45.60±1.83 ab	75.30±2.33 ab	62.72±1.83 a
		15	50.10±1.86 ab	80.00±3.58 ab	57.86±0.97 abcde
Brunello	CMC	0	50.90±2.24 ab	70.70±2.29 ab	53.38±3.54 abcdefg
		2.5	49.10±4.14 ab	68.50±5.34 ab	51.10±2.14 abcdefg
		5	50.90±3.12 ab	69.20±4.53 ab	50.12±1.74 abcdefg
		10	52.60±4.01 ab	72.30±5.27 ab	53.76±1.81 abcdef
		15	49.50±1.90 ab	66.80±2.81 b	49.34±0.50 bcdefg
	MNCC	0	50.90±2.24 ab	70.70±2.89 ab	53.38±3.54 abcdef
		2.5	54.50±2.23 ab	77.30±1.72 ab	49.70±0.61 bcdefg
		5	49.90±2.14 ab	69.80±2.18 ab	51.84±1.78 abcdefg
		10	50.90±3.60 ab	69.60±3.14 ab	51.60±1.68 abcdefg
		15	52.80±3.18 ab	71.80±3.99 ab	49.22±2.08 cdefg
ANOVA (3-way)					
A			**	**	**
B			NS	NS	NS
C			NS	NS	NS
A×B			NS	NS	NS
A×C			NS	NS	*
B×C			NS	NS	NS
A×B×C			NS [Y]	NS [Y]	NS [Y]
CV (%)			10.46	8.68	8.58

N=5. The means with similar letters have not any significant differences at $HSD_{0.01}$.

[W] $HSD_{0.05}$

[Y] Type I Error

**, *, NS = Significance at p≤0.01 and p≤0.05 and non-significance, respectively.

pared to other treatments. Total length showed similar reaction with vegetative length to treatments but the least value was observed in Brunello treated by 15 mg/l CMC. However, *chl* intensity under effects of treatments showed the most value in Navona treated by 10 mg/l MNCC and the least were observed in Cherbourg treated by 10 mg/l MNCC (Table 1). It seems that morphological characteristics of cultivars treated by two types of OSFs in different concentrations are under dominant effect of cultivar; on the other hand, morphological characteristics of different lily cultivars show the various reactions to OSFs and their concentrations. Generally, from the above observations on vegetative development, it can be interpreted that firstly, size of the plant is affected by genotype, and secondly, magnetism may increase the synthesis of *chl* and *chl* density, but it does not affect the size of the shoots and this needs to be investigated more with higher concentrations.

Nguyen Van *et al.* (2013) stated that chitosan nanoparticles can easily penetrate into plant cells and increase the biological activity of cells. They pointed to an important effect of chitosan nanoparticles on the biophysical properties of the coffee plant such as increasing *chl* content and plant growth and development. Răcuciu and Creangă (2007 b) used low-density (less than 100 µl/l) ferrofluids without electromagnetic treatment or any other magnetic field for corn seedlings. They observed that due to their super-paramagnetic effects (a special effect of magnetic nanoparticles) on the structure of photosynthetic enzymes, growth was enhanced. Răcuciu and Creangă (2007 a, b) and Răcuciu *et al.* (2009) used two fluids of magnetic nanoparticles of Fe_3O_4 coated with or without tetraethylammonium hydroxide in Petri dishes for germination of corn seeds without the use of a magnetic field (50 µl per liter). They observed that plant height increased, but when the concentration increased, toxicity was reported. They referred that magnetic iron is a source for plant growth. In addition, magnetism has a good effect on photosynthesis. El-Sayed (2014) reported the increasing effect of magnetic water treatment on plant growth of *Vicia faba*, *chl a* and *b*, and carotenoids, and contents of gibberellic acid and kinetin It is known that kinetin (cytokinins) is effective in preservation and prevention of the chlorophylls destruction (Taiz and Zeiger, 2002).

Farouk and Amany (2012) reported an increase in *chl a* and total *chl* by spraying the chitosan (250 mg/l) on chickpea compared to low concentrations (50 and 125 mg/l), and their interpretation about increasing the content of photosynthetic pigments was the increase of cytokinin level and stimulation of the synthesis of chlorophyll, and they interpreted this as the role of amino groups in chitosan on the mentioned processes. Chlorophyll density below 40 (Spad index) indicates a disruption to photosynthetic process (Netto *et al.*, 2005). However, health of the photosynthetic apparatus in all treatments, especially high concentrations of two OSFs (magnetic and non-magnetic) can be seen. Răcuciu *et al.* (2009) reported that when concentration of Fe_3O_4 magnetic nanofluid was increased up to 300 µl/l, content of *chl a* decreased by about 20 mg/l of fresh weight. In addition, Răcuciu and Creangă (2007 b) stated that the magnetic nanofluid of Fe_3O_4 coated with tetramethylammonium hydroxide (50 µl per liter) produced more pigments (*chl a* and *b* and carotenoid), and higher concentrations of fluids destroyed the photosynthetic process. Limpanavech *et al.* (2008) reported that chitosan affected the expression of chloroplast genes in *Dendrobium* orchid and altered the chloroplast size. Dzung *et al.* (2011) sprayed chitosan at appropriate concentrations on coffee seedlings and reported an increase in content of chlorophyll and also in uptake of mineral elements (nitrogen, phosphorus, potassium, calcium and magnesium), and enhancement of photosynthesis as well as the synthesis of chlorophyll of leaves.

As can be seen in Table 2, 3-way interactions between cultivars, OSFs and their concentrations in ANOVA test due to Type 1 Error could not affect reproductive development of lily plants. However, mean comparisons showed significant effects at $HSD_{0.01}$ on these characteristics. In Cherbourg, the most inflorescence lengths were observed in the plants treated with 15 mg/l MNCC, but in Navona without significant different with Cherbourg, the most lengths of inflorescence was observed in control and 10 mg/l CMC. The shortest length of inflorescence was observed in Brunello. However, this cultivar with the least concentration of magnetic OSF had the most length and generally, it seems that on this characteristic, the effect of genotype was dominant toward OSFs and their concentrations. How to make a reaction of a plant as flower bud number affected by 3-way interactions of factors shows dominant effects of genotype toward OSFs and their concentrations. We can statistically analyze each cultivar separately to observe 2-way interactions and/or single effects of OSFs on the responses of lily plants. Cherbourg and Navona could not show response to OSFs and their concentrations for bud number.

However, Brunello reaction to OSFs and their concentrations was obvious. It can be said that this cultivar could respond to the OSFs compared with two other cultivars, because produced the most bud number with 2.5 mg/l of magnetic compound. In the case of bud length, although 3-way interaction showed significance, it can be seen for this morphological characteristic that the effect of genotype was dominant again, and Cherbourg produced larger buds compared with two others. Furthermore, this response was observed about flowering date, and Cherbourg flowered later (Table 2). From the observations of the effects of three factors, including cultivar, OSFs and concentrations of them, it can be interpreted that this range of the tested concentrations could not affect vegetative and reproductive morphology, and it seems that more concentrations of OSFs must be investigated in future research.

It is known that one of the factors that contribute to growth of the reproductive part (flower bud length) is potassium. Amino groups in the carboxymethylated chitosan and its derivatives can

Table 2 - Effects of OSFs on reproductive development of three lily cultivars

Cultivars (A)	Treatments		Inflorescence length (cm)	Bud No.	Bud length (mm)	Flowering date (days)
	OSFs (B)	Concentration of OSFs (mg/l) (C)				
Cherbourg	CMC	0	28.08±0.56 abc	6.80±0.58 abc	116.63±0.24 a	94.60±0.24 a
		2.5	26.88±0.84 abcdef	6.80±0.37 abc	116.31±0.77 a	93.00±0.77 a
		5	26.90±1.57 abcdef	6.20±0.20 b	123.27±0.49 a	93.20±0.49 a
		10	27.50±1.47 abcd	7.00±0.32 abc	116.14±0.55 a	92.5±0.55 a
		15	27.30±1.87 abcde	6.40±0.40 abc	118.64±0.37 a	93.80±0.37 a
	MNCC	0	28.08±0.56 abc	6.80±0.58 abc	116.64±0.24 a	94.60±0.24 a
		2.5	26.70±1.09 abcdef	6.40±0.51 abc	122.06±0.51 a	93.60±0.51 a
		5	28.40±1.41 ab	6.80±0.37 abc	120.39±0.58 a	93.80±0.58 a
		10	27.48±0.88 abcd	6.80±0.37 abc	110.43±0.68 a	93.40±0.68 a
		15	31.36±2.06 a	7.00±0.58 abc	119.86±0.81 a	94.40±0.81 a
Navona	CMC	0	32.90±1.61 a	9.00±0.32 a	82.87±0.68 b	59.40±0.68 b
		2.5	30.40±0.53 ab	9.50±0.29 ab	80.02±0.73 b	60.20±0.73 b
		5	31.00±2.26 ab	9.20±0.49 a	79.80±0.49 b	59.80±0.49 b
		10	31.60±1.35 a	9.00±0.63 a	81.16±0.63 b	59.00±0.63 b
		15	29.80±1.11 ab	9.40±0.24 a	78.72±0.49 b	58.80±0.49 b
	MNCC	0	32.90±1.61 a	9.00±0.32 a	82.87±0.68 b	59.40±0.68 b
		2.5	30.30±0.89 ab	9.20±0.97 a	76.04±0.58 b	59.20±0.58 b
		5	30.70±1.07 ab	9.00±0.32 a	77.69±0.60 b	59.60±0.60 b
		10	29.70±1.07 ab	10.00±0.71 a	78.95±1.02 b	59.20±1.02 b
		15	29.90±2.34 ab	8.40±0.68 a	80.34±0.60 b	59.60±0.60 b
Brunello	CMC	0	19.80±1.41 cdefg	5.40±0.60 c	78.91±0.97 b	60.20±0.97 b
		2.5	19.40±1.37 defg	5.80±0.97 b	86.39±1.47 b	61.60±1.47 b
		5	18.30±1.52 g	5.80±0.66 b	79.46±1.71 b	62.20±1.71 b
		10	19.70±1.38 defg	5.80±0.97 b	80.04±1.56 b	62.80±1.56 b
		15	17.30±1.28 g	5.60±1.20 c	84.75±1.38 b	61.00±1.38 b
	MNCC	0	19.80±1.41 cdefg	5.40±0.60 c	78.91±0.97 b	60.20±0.97 b
		2.5	22.80±1.23 bcdefg	6.40±0.75 abc	84.40±1.59 b	62.20±1.59 b
		5	19.90±0.89 cdef	5.40±0.75 c	78.47±1.17 b	60.60±1.17 b
		10	18.70±0.85 fg	4.60±0.24 c	76.75±0.20 b	61.20±0.20 b
		15	19.00±0.96 g	5.50±0.96 b	80.59±1.72 b	61.60±1.72 b
ANOVA (3-way)						
A				**	**	**
B				NS	NS	NS
C				NS	NS	NS
A×B				NS	NS	NS
A×C				NS	*	**
B×C				NS	NS	NS
A×B×C				NS [Y]	NS [Y]	NS [Y]
CV (%)				17.61	6.69	1.63

N=5. The means with similar letters have not any significant differences at $HSD_{0.01}$.

[W] $HSD_{0.05}$

[Y] Type I Error

**, *, NS = Significance at p≤0.01 and p≤0.05 and non-significance, respectively.

serve as a place for the absorption of some metal cations. This compound is used to remove heavy metals and water purification and this shows its chelating role (Chang and Chen, 2005; Chang *et al.*, 2006). Chelating sites of chitosan may be effective in absorbing essential metals ions such as manganese, iron (Fe^{+2} and Fe^{+3}), potassium, magnesium, etc. for plants. The abilities of polymer or oligomer chitosan to stimulate plant growth under in vitro conditions; *Vitis vinifera* L. (Barka *et al.*, 2004); orchid *Dendrobium phalaenopsis* (Nge *et al.*, 2006); and the growth of protocorm like bodies in the *Dendrobium* orchid (Pornpienpakdee *et al.*, 2010) have already been reported. Pornpienpakdee *et al.* (2010) used chitosan macromolecules, which had been deacetylated up to 70%, at a concentration of 10 mg/l under *in vitro* culture conditions for *Dendrobium* orchid and produced plants with more length. In addition, Limpanavech *et al.* (2008) when used the polymer or oligomer chitosan (with deacetylated degrees of 70, 80 or 90%) at concentrations of 1-100 mg/l, they observed that polymers at concentrations of 1-10 mg/l and oligomers at concentrations of 50-100 mg/l resulted in more inflorescences production. Gornik *et al.* (2008) applied a type of commercial compound of chitosan, Biochikol 02.PC containing 2% chitosan, at concentration of 0.5% on grape cuttings and reported that root system expanded and it increased the number of new branches and their lengths. Radhakrishnan and Kumari (2012) reported an increase in leaf number and growth of soybean, and an increase in pod length and grain weight under the influence of pulsed magnetic field. El Sayed (2014) reported that magnetic water treatment increased the sink yield in bean (seed and number of seeds per plant). He suggested this effect may be due to increased photosynthetic function of the plant under influence of magnetic treatment. Ohta *et al.* (1999) reported an increase in the photosynthetic reservoir (flower number) in lisianthus with plant seed treatment by chitosan acid solution. Limpanavech *et al.* (2008) also demonstrated that mechanism of chitosan effect on increasing the number of plant photosynthesis reservoirs (number of flowers) in the *Dendrobium* orchid may be caused by effect on the development of photosynthetic apparatus and increasing the size of chloroplasts after chitosan spraying. Kananont *et al.* (2010) reported that different types of chitosan as poly- and oligosaccharides with varying degrees of deacetylation at a concentration of 10 mg/l are effective for *in vitro* culture medium of seed germination of *Dendrobium* orchids and growth of the protocorm like bodies. They stated that chitosan amino groups may be effective factor on the growth of the protocorm like bodies. Pornpinpakdee *et al.* (2010) described role of concentration of different types of chitosan on growth rate of orchid *Dendrobium*.

The results of 3-way ANOVA (Table 3) show significant differences in content of soluble carbohydrates of terminal bud of inflorescence in three cultivars of lily treated with OSFs and their concentrations. The highest content of glucose was significantly observed in Brunello treated with 15 mg/l OSFs regardless of their magnetic or non-magnetic properties. Navona was in second rank in response to treatments compared to Brunello. However, Cherbourg under effects of OSFs and their levels showed the least glucose content regardless of being magnetism or not compared with two other cultivars. Fructose synthesis in the terminal bud had different reaction to the type of OSFs based on genotypes. Generally, the most concentrations of OSFs regardless of their structures had the most effect on fructose content (Table 3). Sucrose approximately had similar response to glucose toward treatments, but about this carbohydrate after 'Brunello', 'Cherbourg' had second score for the most content not 'Navona'.

Starch and other sugars and proteins (enzymes) are photosynthetic products that are transported to storage organs (flowers, seeds or bulbs). Iron acts as a cofactor for many enzymes, forming part of cytochromes and involves in biochemical reactions, including respiration, photosynthetic material transfer, nitrate synthesis, nitrogen fixation, and DNA synthesis (He *et al.*, 2011). El Sayed (2014) reported that photosynthetic function of bean plant increases with magnetic water treatment. He reported that contents of glucose and sucrose as well as polysaccharides in the leaves, stems and the whole plant of bean were higher toward irrigation without magnetic field. In addition, positive effect of chitosan on plant growth may be due to its effect on increasing the phosphorus content. Phosphorus is an essential element in the biosynthesis and carbohydrate transfer for cell division and the formation of DNA and RNA (Farouk and Amany, 2012).

According to Table 4, variance analysis of OSFs effects (type and concentration) on the α-amylases activities of three cultivars of lily showed the significance of 3-way interactions. OSFs effects on α-amylase in 'Navona' compared with two other cultivars showed the highest activity of this enzyme in the highest tested concentration of non-magnetic OSF.

Table 3 - Effects of OSFs on soluble carbohydrates into the terminal bud tissue of inflorescence in three lily cultivars

Cultivar (A)	Treatments		Glucose	Fructose	Sucrose
	OSFs (B)	Concentration of OSFs (mg/l) (C)			
Cherbourg	CMC	0	0.80±0.11 g	2.20±0.13 ghij	3.21±0.19 ghi
		2.5	1.95±0.24 fg	2.21±0.18 ghij	5.70±0.28 def
		5	3.54±0.18 de	3.34±0.17 efg	8.21±0.35 ab
		10	4.50±0.23 bcd	3.33±0.20 efgh	8.19±0.28 abc
		15	3.50±0.24 de	4.61±0.21 bcd	5.70±0.23 def
	MNCC	0	0.80±0.11 g	2.20±0.13 ghij	3.21±0.19 gh
		2.5	0.89±0.13 g	3.33±0.16 efghi	5.71±0.24 def
		5	1.98±0.23 fg	3.27±0.18 efghi	5.67±0.15 def
		10	3.50±0.11 de	4.60±0.28 bcd	8.23±0.28 ab
		15	2.01±0.29 g	5.51±0.23 ab	8.22±0.44 ab
Navona	CMC	0	2.64±0.22 ef	2.81±0.18 fghi	1.53±0.26 i
		2.5	2.66±0.24 ef	4.03±0.24 cde	3.03±0.20 hi
		5	3.49±0.22 de	4.03±0.20 cde	3.07±0.19 hi
		10	5.03±0.31 abc	4.83±0.26 bc	4.53±0.25 fgh
		15	5.02±0.28 abc	6.05±0.31 a	5.81±0.17 de
	MNCC	0	2.64±0.22 ef	2.81±0.18 fghi	1.53±0.26 i
		2.5	3.51±0.23 de	1.54±0.13 jk	1.59±0.15 i
		5	5.02±0.32 abc	2.60±0.11 fghij	3.10±0.16 ghi
		10	5.84±0.23 ab	2.75±0.19 fghi	3.06±0.19 hi
		15	5.88±0.23 ab	4.02±0.17 cde	4.49±0.23 fgh
Brunello	CMC	0	1.54±0.17 fg	0.82±0.12 k	4.73±0.23 fgh
		2.5	2.72±0.18 ef	0.83±0.14 k	6.80±0.34 bcd
		5	4.03±0.25 cde	2.12±0.14 ij	06.80±0.31 bcd
		10	4.81±0.23 abcd	3.65±0.19 cdef	9.81±0.41 a
		15	6.03±0.30 a	3.57±0.19 def	9.84±0.46 a
	MNCC	0	1.54±0.17 fg	0.82±0.12 k	4.73±0.23 efg
		2.5	4.02±0.23 cde	0.88±0.18 k	4.81±0.24 efg
		5	4.81±0.25 abcd	2.13±0.18 hij	6.46±0.19 cde
		10	4.84±0.21 abcd	3.58±0.20 def	9.82±0.40 a
		15	6.02±0.26 a	4.70±0.25 bcd	9.79±0.36 a
ANOVA (3-way)					
A			**	**	**
B			NS	**	**
C			**	**	**
A×B			**	**	**
A×C			**	**	**
B×C			NS	NS	**
A×B×C			**	**	**
CV (%)			10.88	10.28	7.96

N=3. The means with similar letters have not any significant differences at $HSD_{0.01}$.
[W] $HSD_{0.05}$
[Y] Type I Error
**, *, NS = Significance at p≤0.01 and p≤0.05 and non-significance, respectively.

While, the highest activity recorded for this enzyme in ‘Brunello’ was observed in magnetic OSF (MNCC) unlike ‘Navona’. Cultivar Cherbourg unlike two other cultivars showed the highest activity of α-amylase regardless of being magnetic of OSF with the high concentrations of two OSFs, in tissue of the last flower bud of inflorescence. Therefore, responses types of cultivars toward being magnetic or not of OSFs were different. On the other hand, the amount of activity of this enzyme in ‘Navona’ was higher regardless of the type of OSF and their concentrations.

According to 3-way ANOVA, β-amylase activity in tissue of the terminal flower bud of inflorescence was significantly affected by three factors (Table 4). The activity of this enzyme unlike α-amylase was more in ‘Brunello’, and like α-amylase, the activity of this enzyme was more with the highest concentration of magnetic OSF. The β-amylase activity in ‘Cherbourg’ was like α-amylase in the same cultivar. However, in ‘Navona’, this enzyme showed the highest activity affected by the both of magnetic and non-magnetic OSFs with different manner toward α-amylase in the same cultivar.

The Earth's magnetic field affects orientation of the ferromagnetic particles and modulations of reactions. It has been reported that the magnetic field has an effect on the biochemical processes and stimulation of the activity of proteins and enzymes on increase of the seed vigor (Dhawi *et al.*, 2009). Tham *et al.* (2001) reported an increase in shoot growth of rice seedling in the hydroponic medium using acid solution of chitosan. Magnetic fields have been reported to increase sugar content in sugar beet roots (*Beta vulgaris*) and the content of gluten in wheat (*Triticum aestivum*) (Dhawi *et al.*, 2009). Radhakrishnan and Kumari (2012) in their experiment on soybeans indicated a positive effect of a pulsed magnetic field on the increased activity of the α- and β-amylases. In our experiment, MNCC with its synergetic effects of magnetism, chitosan, and iron, was remarkably increased the photosynthetic structures,

Table 4 - Effects of OSFs on amylases (N=3) into the terminal bud tissue of inflorescence and vase life (N=5) of three lily cultivars

Cultivars (A)	Treatments		α-amylase	β-amylase	Vase life (days)[wz]
	OSFs (B)	Concentration of OSFs (mg/l) (C)			
Cherbourg	CMC	0	4.48±0.21 gh	5.98±0.49 g	7.00±0.00 ab
		2.5	4.42±0.21 gh	7.97±0.30 g	8.20±0.97 ab
		5	6.61±0.33 ef	12.31±0.90 de	7.00±0.71 ab
		10	6.61±0.20 ef	13.49±0.68 d	7.20±0.37 ab
		15	9.00±0.32 cd	12.33±0.99 de	6.00±0.63 ab
	MNCC	0	4.48±0.21 gh	5.98±0.49 g	7.00±0.00 ab
		2.5	6.56±0.21 ef	6.01±0.43 g	6.60±0.51 ab
		5	7.14±0.17 def	8.04±0.30 g	6.67±0.67 ab
		10	8.99±0.49 cd	12.04±0.95 def	8.00±0.00 ab
		15	11.07±0.44 ab	8.07±0.33 g	6.40±0.51 ab
Navona	CMC	0	5.60±0.27 fg	9.06±0.36 efg	7.80±0.37 ab
		2.5	8.09±0.40 cde	9.11±0.45 ef	7.20±0.49 ab
		5	8.02±0.41 cde	12.15±0.63 de	7.80±0.20 ab
		10	9.63±0.34 bc	15.21±0.65 cd	8.60±0.51 a
		15	12.04±0.53 a	15.11±0.73 cd	8.20±0.49 ab
	MNCC	0	5.60±0.27 fg	9.06±0.36 efg	7.80±0.37 ab
		2.5	3.07±0.20 hi	12.10±0.50 def	6.80±0.37 ab
		5	5.57±0.22 fg	14.92±0.68 cd	8.80±0.58 a
		10	5.59±0.27 fg	18.09±0.79 abc	7.80±0.37 ab
		15	8.03±0.34 cde	18.13±0.74 abc	9.25±0.48 a
Brunello	CMC	0	2.04±0.14 i	6.52±0.37 g	5.80±0.58 ab
		2.5	2.18±0.12 i	8.16±0.44 fg	4.80±0.73 b
		5	4.27±0.24 gh	13.38±0.53 d	6.20±0.37 ab
		10	6.82±0.32 ef	15.27±0.75 cd	7.00±0.77 ab
		15	6.81±0.35 ef	19.30±0.69 ab	7.67±0.67 ab
	MNCC	0	2.04±0.14 i	6.52±0.37 g	5.80±2.58 ab
		2.5	2.09±0.07 i	12.99±0.61 de	6.20±0.20 ab
		5	4.18±0.22 gh	15.43±0.81 bcd	7.60±0.51 ab
		10	6.80±0.24 ef	15.11±0.70 cd	5.80±1.02 ab
		15	9.29±0.50 bc	19.41±0.79 a	5.80±1.02 ab
ANOVA (3-way)					
A			**	**	*
B			**	*	NS
C			**	**	NS
A×B			**	**	NS
A×C			**	**	NS
B×C			**	**	NS
A×B×C			**	**	NS[y]
CV (%)			7.84	7.99	19.18

[z] The means with similar letters have not any significant differences at $HSD_{0.01}$.
[w] $HSD_{0.05}$ and N=5
[y] Type I Error
**, *, NS = Significance at p≤0.01 and p≤0.05 and non-significance, respectively.

the transfer of photosynthetic products and the storage of carbohydrates; so that Takeda *et al.* (1983) stated that the increase of substrate promotes the activity of amylase enzymes, especially α-amylase.

According to Table 4, the longer vase life was observed in 'Navona' in the high concentrations of OSFs (magnetic and non-magnetic) based on 3-way interactions compared with two other cultivars. Hajnorouzi *et al.* (2011) reported that using a combination of Earth's magnetic field and weak pulsed electromagnetic field on corn seedlings can increase the growth rate and decrease the iron content of the plant and maintain the membrane's health and decrease oxidative burst.

Since the magnetic field is the natural property of the earth, plants and other living creatures are permanently responding to the magnetic field during their lives. Earth acts as a magnet with its northern and southern poles, and natural effects of the magnetic field can change the growth and yield of plants on the ground. In particular, the electromagnetic spectrum of solar radiation stimulates plant growth through the process of photosynthesis. The possible mechanism is the changing of the electrostatic balance of the plant system at the membrane surface of the cell, which is the primary site for any plant growth restriction or promotion (Radhakrishnan and Kumari, 2012). Therefore, the role of superparamagnetic nanoparticles can be interpreted in our experiment based on the influence of the Earth's magnetic field.

It can be stated, instead of the iron salt (which is often difficult to uptake by plants), a chelating agent in the nutrition solution can be used both as the iron source for the plant and also is suitable for the absorption of other elements. Between two tested compounds, MNCC is a more suitable compound than CMC because it has a positive synergic effect in addition to providing iron in the plant, which is effect of both chitosan and magnetism. In addition, the characteristics of particle size in this type of supplement, i.e. nano- size, affect the biophysical characteristics and biological activities of the plant.

4. Conclusions

Our experiment showed that utilizing the magnetic composite of chitosan (MNCC) or modified and chelating macromolecule of chitosan (CMC) in horticulture can significantly affect the growth and development physiology of plants. We introduced this compound, especially magnetic compound, as fertilizer supplement in production period of lily bulb (Shafiee-Masouleh *et al.*, 2014); now in this experiment for three cultivars forcing period of lily shows that high concentration of both OSFs can be used as a supplement in nutrition solution. The highest concentrations (10 and 15 mg/ l) regardless of OSF types and the cultivars response caused significant physiological effects on the content of carbohydrates and also the enzymes that are influence on carbohydrates (amylases). It seems that observing the remarkable morphological changes needs to use the higher concentrations of both OSFs without toxic effects. However, this subject (the use of higher concentrations) must be examined in horticultural plants. The examined cultivars (Cherbourg, Navona and Brunello) showed almost different responses to OSFs, but generally magnetic OSF can be advised and for security utilization needs to be examined with higher concentrations at future research.

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