INFLUENCE OF CONTROLLED RELEASE FERTILIZERS ON Lilium regale SPECIES GROWTH AND FLOWERING

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Abstract

Our research aims to establish the influence of Osmocote®Pro fertilizer on ornamental characteristics, photosynthetic pigments content, superoxide dismutase and peroxidase activities for Lilium regale species. The tests were organized in four variants, each with three replicates, each replicate consisting of 10 bulbs. The growth of plants in the four experimental variants was achieved without fertilization in case of the control variant (M) and under fertilization conditions with Osmocote®Pro for the rest of variants (V1 - 4g fertilizer/plant; V2 - 6g fertilizer/plant and V3 - 8g fertilizer/plant). The influence of fertilization on plant growth and development has been assessed by plant height, number of flowers, cups’ diameter and length. The growth in height and capacity of plant flowering were stimulated by applying the fertilizer, regardless of the dosage used. The application of fertilization with Osmocote®Pro at Lilium regale species caused an increase in plant height, number of flowers per plant, cup diameter, the best results being highlighted for the variant at which we have applied the highest dose of fertilizer. Compared with the control, the increasing of the fertilizer concentration determined a further growth in terms of plant height, number of flowers, diameter and length of the cups. At the physiological level, in the fertilized variants we observed an increase in the total content of assimilating pigments, the content of chlorophyll a, b. The results pointed out that plants fertilization was positively correlated with the enzymatic activity, while a decrease of superoxide dismutase activity compared to the control was observed.

Keywords: fertilizer, Lilium regale, photosynthetic pigments, SOD

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1. Introduction

The Lilium genus includes species that are considered to be excellent for use as cut flowers, which grants this species 4th place in the global trade (Raj, 2015; Zhao et al., 2018). It is considered to be one of the genera of great economic importance in the production and marketing of cut flowers on the international market (Jimenez et al., 2012). Lilies are floral plants with a long history and they maintained their position as one of the most important group of ornamental plants, being of great value for landscape design projects, pot plants and, not lastly, cut flowers (Pelkonen and Pirttilä, 2012; Tredter, 2005). In present, Lilium is one of the top ten superior cut flowers in the world due to its morphological particularities: the size, color, perfume and longevity of the floral cups (Thakur et al., 2005). As cut flowers, this genus is highly appreciated and it is among the most beautiful and elegant flowers, which makes it popular for different occasions. In order to produce high quality lily plants, growers need to identify the optimal

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nutritional requirements and the difficulties that arise during the production process (Barnes et al., 2011).

Currently, as a consequence of rising market requests for this species, there is a wide range of new varieties of lilies. *Lilium regale* is one of the most important plants used for its cut flowers, it is part of the rustic geophytes and it has several origins centers such as Asia, America and Europe (Kumar et al., 2007). Due to its intense and highly appreciated perfume and the special ornamental value of its calyx, the plant gained the name of imperial lily and it became increasingly more requested for cut flowers. In the process of field-cultivation of lilies for cut flowers, during spring and summer it is important to consider the landscape zone (Gonz lez Hurtado et al., 2015; Tolesu et al., 2010). In the cut flowers industry, quality is one of the most important criteria and it is only determined by the application of fertilizers. In the case of the *Lilium* flower crops for cut flowers, the full supply of micronutrients and macronutrients in adequate quantities and appropriate proportions is one of the most important factors that control plant growth (Draghia and Chelarian, 2011; Giri et al., 2018). Due to the importance of fertilization on the quality of flowers, the fertilization with controlled release fertilizers (CRF) is now widely used due to the efficiency of nutrient utilization, better plant growth and quality, reduction of mineral nutrient loss, compared to the use of quick release fertilizers (Cabrera, 1997; Harris et al., 1997; Husby et al., 2003; Wilson and Albano, 2011).

The release of nutrients from CRF is mainly influenced by temperature and time, higher temperatures generally generating an increase in the nutrient release rate. The longevity of the release of macro and microelements in the case of the controlled release fertilizers can be defined as the length of time in which a fertilizer releases a high percentage of nutrients of about 80% (Matysiaik and Nogowska, 2016).

Some studies demonstrated that the use of Osmocote with controlled release was much more efficient than ferti-irrigation, since the use of this fertilizer reduces the loss of N, P, K (Andersen et al., 2000; Broschat, 1995; Segura et al., 2007) as well as it avoids environmental pollution caused by the leakage of dangerous substances for the environment into the aquifer (Huett and Morris, 1999; Mikkelsen et al., 1994; Schroeter-Zakrzewska and Kleiber, 2012).

In some species, an improved level of fertilization has been achieved by studying the influence of some slow release fertilizers on the ornamental characteristics of different ornamental species (Bosiacki, 2008; Cieciora et al., 2006; Kozik et al., 2004; Schroeter-Zakrzewska and Kleiber, 2012; Zurawik and Placek, 2011). The wide use of this type of fertilizer in flower cultures meant for the commercialization as cut flowers or pot plants was due to the practical aspect consisting in simplifying the cultivation methods but mostly on the quality of the plants obtained (Bosiacki, 2008; Startek, 2002). The novelty of our work is highlighted by the use of the controlled release fertilizer (Osmocote) to obtain *Lilium regale* cut flowers in crops grown in...
Influence of controlled release fertilizers on Lilium regale species growth and flowering

experimental fields. Given this context, we propose a series of experiments developed to show the importance of controlled release fertilizers to increase the productivity and quality of the cut flowers from Lilium regale species.

2. Materials and methods

The experiments were conducted using bulbs of Lilium regale supplied by a firm specialized in the commerce of floral seedling from Netherland (Everris®). Osmocote® Pro is a controlled release fertilizer from the 2nd generation produced by Everris® and purchased from S.C. Morami S.R.L., Salonta (Romania). It has a high content of NPK, magnesium and all microelements in concentrations of N11:P10:K19:2MgO+ME. It has a very regular release pattern and its longevity of up to 5-6 months. Osmocote® Pro is especially suitable for use as a basic fertilizer mixed in the culture substrate. Osmocote® Pro fertilizer was used in our experiments due to its regular pattern of release and its longevity of up to 5-6 months.

The whole experiment consisted of 4 groups of variants, as follows: M variant was designated to be the control variant and was performed without any fertilizer application, V1 variant consisted in the application of 4g of Osmocote/plant, V2 variant benefitted of 6g of Osmocote/plant, while V3 variant in 8g of Osmocote/plant. The studies were conducted during 2013 – 2016 and the observations, measurements and analysis were performed on four sets of samples from each experimental variant. Each year a new set of samples was realized. In order to establish the influence of the fertilizer Osmocote® Pro on the growth of Lilium regale, we conducted biometric determinations of the height of the floral stems, the number of flowers on the plant, the length and the diameter of the flowers.

In addition to the biometric determinations, physiologic analyses were conducted in order to determine the content of assimilating pigments and the activity of the superoxide dismutase in the mature leaves. Both sets of samples were analyzed twice a year, in June and July, during 2013-2016.

The extraction and determination of assimilating pigments were conducted in accordance with the Current Protocols in Food Analytical Chemistry (Lichtenthaler and Buchmann, 2001). The tissues of the fresh leaves (0.1 g) have been mortared in acetone (pure solvent) and then centrifuged at 10,000 rpm for 5 minutes. After centrifugation, the reading of the supernatant was performed at the absorbance 661.6 nm for chlorophyll a (Chl. a), 644.8 nm for chlorophyll b (Chl. b) and 470 nm for carotenoids (car.), using T70 UV/VIS Spectrophotometer PG. In order to determine the activity of the superoxide dismutase, the pulverization of the leaves was conducted on ice in phosphate pad/buffer with pH of 10.2. After the pulverization, the extract was spun at 12,000 rpm for 15 minutes at 4°C. The determination of the activity of superoxide dismutase (SOD) was conducted using the colorimetric method with xanthine oxidase and the kit was produced by Foortress Bioclinical (Great Britain). The extinctions were measured at a wavelength of 505 nm using a semi-automatic biochemical analyzer, model Cormay Multi (Poland, 1991).

The determination of peroxidase activity was done using the o-dianisidine colorimetric method (L. V. Gudkova and R. G. Degtiari method, after Artenie et al., 2008). The method focuses on measuring the color intensity of the o-dianisidine oxidation product with hydrogen peroxide, under the action of peroxidase. In order to obtain the enzymatic extract, 0.1-0.5 g of vegetable tissue are ground and added to the same mass of crushed glass or quartz sand. The ground tissue is quantitatively transferred into 3 mL of 0.1M disodium phosphate buffer solution for extraction. The grinding is done in an ice bath. The obtained homogenate is centrifuged for 15 minutes at 3,000 rpm in a refrigerated centrifuge. The separate supernatant is used as the source of the enzyme. At the time of the determination of peroxidase activity, in a 20 mL test tube, a 0.1 - 0.5 mL enzymatic extract (supernatant), 3 mL buffer solution (reagent 5) are measured and bidistilled water at 4, 8 mL is added. 0.2 mL of 0.05% hydrogen peroxide solution is added and the tube is immersed in a 20°C water bath for exactly 5 minutes. The action of peroxidase is stopped by adding 5 mL of 50% H2SO4 solution. The color intensity is determined with a spectrophotometer using the 540 nm wavelength and 10 mm-wide cuvettes, against a control sample containing the following components of the reaction system, added in the following order: bidistilled water (4.7-4.3 mL), 50% sulfuric acid solution (5 mL), enzymatic extract (0.1 - 0.5 mL) and 0.05% hydrogen peroxide solution (0.2 mL). The action of peroxidase is calculated by the value of the micromolar extinction coefficient, equal to 0.0128. A unit of peroxidase (UP) corresponds to the amount of enzyme catalyzing the decomposition of one micromole of H2O2 in one minute under optimum conditions. The number of peroxidase units (UP) per g of tissue is calculated using Eq. (1):

$$UP = \frac{E_P}{0.0128 \cdot \frac{1}{1000} \cdot \frac{1}{5} \cdot \frac{10}{v} \cdot \frac{1}{p}}$$ (1)

where, $E_P$ is the extinction of the sample read on the spectrophotometer; 0.0128 represents the value of the micromolar extinction; 1000 represents the number of mL in a l; 5 is the total sample volume (mL); v is the volume of peroxidase extract (supernatant, in mL) introduced into the reaction environment; and p is the amount of vegetal tissue in g.

All tests were conducted in the Cultivating Field of the Floriculture Discipline that is part of the Vasile Adamaachi teaching farm within the University of Agricultural Sciences and Veterinary Medicine in Iasi.
3. Results and discussion

3.1. Influence of fertilization on growth and development of plants

The biometric determinations on the medium height of the floral stems showed a positive influence of fertilization on the growth of plants from the Lilium regale species. The results obtained in the study also showed that the concentration of fertilizer influences the growth and development of plants.

As compared to the plants from the control variant (where no Osmocote has been applied), in all the other experimental variants the growth of the plants has been directly proportional with the increase of the fertilizer dosage. As compared to the control variant, the increase in height was of 6.9 cm for the plants included in V1 variant, of 21.1 cm in V2 and 35.5 cm in V3 variant (Table 1).

The fertilization with Osmocote also had a positive influence on the formation and development of flowers in the Lilium regale species, the results obtained in the study showing an increase in the number of flowers on the plants in all fertilized variants (Table 1). Considering the tendency of increasing number of flowers on each plant, we can notice that the variants that have been fertilized with the highest dosages showed a significant increase as compared with the unfertilized variant.

Thus, the increase of the dosage of Osmocote®Pro in the substrate determined an increase of the number of flowers with 2.3 more flowers in V1 variant as compared to the control variant, 4.1 more flowers for the plants included in V2 variant and 8.2 more flowers for the plants from the V3 group (Fig. 1).

The increase of the concentration of fertilizer produces a slight increase of the length of the flowers as compared to the control variant: 0.8 cm longer flowers in the group of plants from V1 variant, 1.6 cm in the plants from V2 variant and 2.1 cm in the plants from V3 variant. Nevertheless, the diameter of the flowers knew no significant increases in either of the fertilized groups of plants. The studies carried out on lachenalia (Kapczyńska, 2014), hyacinths and lilies, have shown that the size of the bulbs used in planting is important for the development of new bulbs and bulbils (Addai and Scott, 2011). In general, the increase in P and K concentrations influences the vegetative growth (leaf length, width, foliar surface and stem height, flowering, formation and bulb growth) (Varshney et al., 2001). Studies have shown that with the increase in K concentration, the nutrient absorption in the plant increases, which leads to an increase in the dry matter content of flowers and bulbs for Lilium (Choi et al., 2004).

3.2. Influence of fertilization on the formation of new bulbs and bulbils

In order to demonstrate the effect of the controlled release fertilizers, such as Osmocote, determinations have been made regarding the formation of new underground organs for the Lilium regale. To determine the influence of fertilization on the formation of new bulbs and bulbils, the harvesting of the underground storage organs was carried out during the rest period / dormancy period of the plants.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Medium height of plant (cm)</th>
<th>Number of flowers (piece)</th>
<th>Length of the flowers (cm)</th>
<th>Diameter of the flowers (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>85.4±1.97</td>
<td>8.3±0.14</td>
<td>8.2±0.10</td>
<td>6.7±0.63</td>
</tr>
<tr>
<td>V1</td>
<td>92.3±1.71</td>
<td>10.4±0.18</td>
<td>10.3±0.14</td>
<td>7.1±0.98</td>
</tr>
<tr>
<td>V2</td>
<td>106.5±2.31</td>
<td>13.4±0.17</td>
<td>12.4±0.13</td>
<td>8.0±0.78</td>
</tr>
<tr>
<td>V3</td>
<td>120.7±2.02</td>
<td>18.5±0.35</td>
<td>18.5±0.21</td>
<td>8.6±064</td>
</tr>
</tbody>
</table>

Each value is shown as the mean ± S.D.

![Fig. 1. Growth of Lilium regale plant in the experimental field:](image)
As a result of the measurements, there was an increase in the number of newly formed bulbs and bulbils as the fertilizer concentration increased. There has been an increase in the number of bulbs and bulbils starting with V1 variant, which suggests that the Osmocote fertilization has been of particular importance for plant propagation.

By comparing the results obtained in the case of the plants from the three fertilized variants with the results obtained in the case of the plants from the non-fertilized variant (control variant), we may note that the best results were obtained for the plants of V3 variant (Fig. 2). From the graphical representation of the results (Fig. 2), we can observe that in the case of the control variant, there were formed, on average, 2.6 bulbs and no bulbils. As the fertilizer concentration increased, an increase in the number of bulbs was observed, reaching an average of 5.8 bulbs and 7.7 bulbils in the V3 variant. In the V1 and V2 variants, intermediate values were obtained, the plants of V1 variant obtaining an average number of 3.5 bulbs and 2.5 newly formed bulbils, while in the case of V2 variant, the average numbers were 4.7 bulbs and 5.0 bulbils.

In order to achieve this goal, several stages were considered, a set of measurements being made for each variant. The average weight of the mother bulb, the newly obtained bulbs and bulbils was determined within the measurements made. In the case of the four experimental variants, there was a variation observed in the average mass, which depended on the amount of fertilizer administered and on the number of newly formed bulbs and bulbils. From Fig. 3, we may note the average weight of the mother bulb and of the newly formed bulbs and bulbils. In the case of the control variant (M), one year after the establishment of the culture, the plants’ mother bulb had an average weight of 21.50 g, the newly formed bulbs had an average weight of 18.4 g, no bulbils being formed.
In V1 variant, the average weight of the mother bulb was 19.0 g, the newly formed bulbs had an average weight of 103 g and the bulbils had an average weight of 3.5 g. V2 variant had the highest average weight values recorded out of all variants, the average weight of the mother bulb being 26.5 g, in the case of the newly formed bulbs, the average weight being 14.7 g, while the bulbils had a weight of 4.8 g. In the case of the plants from V3 variant, there was a slight decrease in the mass of the mother bulb compared to the plants from V2 variant, the average of the values being of 24.1 g. As for the weight of the newly formed bulbs, they are weighing more than the ones from V3 variant, having an average value of 19.4 g, while the bulbils had an average weight of 4.0 g. These differences are due to the specific consumption that is higher for plants with a large number of newly formed bulbs and bulbils. We may note a decrease in the weight values in V2 variant, due to the higher number of bulbs and bulbils, which leads to a higher specific consumption, hence the result of obtaining underground storage organs with a smaller mass.

### 3.3. Influence of fertilization on the content of assimilating pigments

Another objective of our study was to investigate the influence of fertilization on the content of assimilating pigments and on the activity of the superoxide dismutase (SOD). The experimental results included in Table 2 show differences regarding the total content of assimilating pigments obtained within the four experimental variants. Furthermore, the results obtained in the study showed an increasing trend of the total content of assimilating pigments determined by the increase of the concentration of Osmocote. Within the four variants, the total content of photosynthetic pigments varied from 2.00 mg g⁻¹ DW in the group of plants from control variant (M) to 2.48 mg g⁻¹ DW in the group of plants from V3 variant. When comparing the results obtained in the fertilized variants to those from the unfertilized variant, we could observe an increase of the total content of assimilating pigments: 0.13 mg g⁻¹ DW in the group of plants from V1 variant, 0.36 mg g⁻¹ DW for the group of plants from V2 variant and 0.48 mg g⁻¹ DW in the group of plants from V3 variant.

In general, an increase of the content of chlorophyll \(a\) is noted in all fertilized variants, the values varying from 1.53 mg g⁻¹ DW in the group of plants from V3 variant and 1.11 mg g⁻¹ DW in the group of plants from control variant (M). The content of chlorophyll \(a\) in the control variant was significantly lower than in the case of the fertilized variants; the comparison of results showed increases of 0.16 mg g⁻¹ DW in the group of plants from V1 variant, 0.35 mg g⁻¹ DW in the group of plants from V2 variant and 0.42 mg g⁻¹ DW in the group of plants from V3 variant. The results regarding the content of chlorophyll \(b\) showed values varying from 0.40 mg g⁻¹ DW in the group of plants from the control variant to 0.50 mg g⁻¹ DW in the group of plants from V3 variant.

The content of chlorophyll \(b\) obtained in all fertilized variants was slightly higher, ranging from 0.02 mg g⁻¹ DW to 0.10 mg g⁻¹ DW. The studies conducted by Hendry and Price (1993) demonstrated that the decrease of the content of chlorophyll in the plants is usually associated with the stress caused by the growing condition, while the variation of the ratio between the total content of chlorophyll pigments and carotenoids is a good indicator of the stress caused in the plant. Our studies revealed that controlled - release fertilizing with Osmocote favors the physiological processes in *Lilium regale* for all fertilized variants by obtaining an increase in the photosynthetic pigments content. From a theoretical point of view the ratio chlorophyll \(a+b\)/carotenoid pigments is 4.8:1, while the ratio chlorophyll \(b\)/carotenoids is 1.5:1 (Park and Biggins, 1964). The results of the analyses show that, in the fertilized variants, the ratio between chlorophyll \((a+b)\) and the carotenoid pigments has increased, on average, by 22.58% in the plants from the group V1, by 35.48 % in the group of plants from V2 and by 45.16% in the group of plants from V3. Regarding the ratio chlorophyll \(b\)/carotenoids, the results show a slight decrease of the values below the theoretical limit. The decrease of the values obtained in the study varied from 46% in the group of plants from control variant to 27% in the group of plants from V3 variant.

### 3.4. Influence of fertilization on the activity of the superoxide dismutase and peroxidase activity

Within the experiment, we also determined the activity of superoxide dismutase in the plants from all groups. According to data from literature, the development of plants without the necessary nutritive elements determines not only a diminution of the ornamental characteristics but also a stress at the physiological level causing an intensification of the enzymatic activity (Marques et al., 2014). The fertilization with this controlled - release product provided the necessary nutritive elements for a proper development of plants throughout the entire vegetative period, leading to a significant decrease of the SOD activity in the leaves as compared to the results obtained in the control variant.

The results of the present study confirm those of several studies where a similar reduction of the activity of SOD has been observed in other species where the fertilization was performed using fertilizers with controlled release (Alscher et al., 2002). As compared to the control variant, in all fertilized variants there has been noted a decreased in the activity of SOD by 3.67% in the plants from V1 variant, 34.05% in the plants from V2 variant and 36.53% in V3 variant.
The graphic representation of the results regarding the activity of the superoxide dismutase shows that the percentage varied from 96.33% in the group of plants from V1 variant to 63.47% in the group of plants from V3 variant (Fig. 4). An important element in the development of lilies is potassium due to the fact that this element leads to an optimal improvement in plant growth and prolongs the flowers resistance to vase keeping, helps in the synthesis of proteins and neutralizing the anions (Pardo et al., 2006). This element plays a key role in protein synthesis, photosynthetic processes and material transportation. Since potassium plays an important physiological role in the growth of plants under unfavorable conditions (Cakmak, 2005; Vyas et al., 2001), the authors investigated the effect of a K-rich controlled release fertilizer on the physiological processes of Lilium regale.

In the case of potassium deficiency in the plant, the activity of certain enzymes, absorption and transport of certain nutrients are reduced (Kanai et al., 2007). In the studies carried out by Torkashvand et al. (2018) it was demonstrated that the application of higher K concentrations results in a decrease in the peroxidase and ascorbate peroxidase activity in the Lilium species. The results obtained in our study, considering the four experimental variants of Lilium regale, are in line with the results of the studies carried out so far, observing a decrease in peroxidase intensity in all the variants fertilized with Osmocote (Fig. 5).

Based on the results obtained, we may note that the fertilization done using fertilizers with controlled release helps in the development of the physiological processes, resulting in a more reduced respiration activity. The values obtained from the variants that received the highest Osmocote doses reveal a decrease in peroxidase intensity in all fertilized variants, with the increase in the Osmocote dose. For the the Lilium regale species, compared to the control variant, a decrease in peroxidase intensity is observed in all fertilized variants. The most pronounced decreases compared to the control variant were highlighted by V2 variant (24%) and V3 variant (by 31%). By comparing the results obtained regarding the peroxidase activity with those regarding the superoxide dismutase, the same tendency of decrease in the enzymatic activity, for the fertilized variants, compared to the control variant, was observed.

4. Conclusions

The fertilization with Osmocote® Pro in Lilium regale had a high influence on the main morphologic ornamental characteristics of the species (the increase of both the height of the plants and the number of flowers per plant). A positive influence could also be noticed on the size of the flowers which presented a slight increment in the diameter and in length.

Table 2. Medium content of photosynthetic pigments in the species Lilium regale (mg g⁻¹ DW)

<table>
<thead>
<tr>
<th>Variant</th>
<th>Chlorophyll a mg g⁻¹ (DW)</th>
<th>Chlorophyll b mg g⁻¹ (DW)</th>
<th>x+c mg g⁻¹ (DW)</th>
<th>Σ Chlorophyll a+b/Carotenoids ratio</th>
<th>Chlorophyll b/Carotenoids ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>1.11±0.08</td>
<td>0.40±0.03</td>
<td>0.49±0.06</td>
<td>2.00</td>
<td>3.1:1</td>
</tr>
<tr>
<td>V1</td>
<td>1.27±0.09</td>
<td>0.42±0.04</td>
<td>0.44±0.04</td>
<td>2.13</td>
<td>3.8:1</td>
</tr>
<tr>
<td>V2</td>
<td>1.46±0.10</td>
<td>0.45±0.05</td>
<td>0.45±0.05</td>
<td>2.36</td>
<td>4.2:1</td>
</tr>
<tr>
<td>V3</td>
<td>1.53±0.12</td>
<td>0.50±0.05</td>
<td>0.45±0.06</td>
<td>2.48</td>
<td>4.5:1</td>
</tr>
</tbody>
</table>

Each value is shown as the mean ± S. D.; DW-dry weight.

Fig. 4. Influence of fertilization on the activity of superoxide dismutase in Lilium regale (% of the control variant)
Fig. 5. Influence of fertilization on peroxidase activity in Lilium regale leaves [UP/(g·min)]

The number and average mass of bulbs and bulbils newly formed on the plant, for the fertilized variants, increased with the increase of Osmocote concentration, the highest values being recorded in the plants from the variant fertilized with the highest concentration (V3 variant, 8g / plant).

The influence of the fertilization has also been observed on physiological level, as the results of the study showed an increase of the content of chlorophyll a and b and the total content of photosynthetic pigments in all plants from the groups of fertilized variants. In all fertilized variants, the fertilizer with controlled - release and longevity of 5-6 months determined a decrease of the activity of superoxide dismutase and the activity of peroxidase as compared to the control variant.

Considering that the plant is mainly cultivated to be valued as a cut flower, the results obtained in the study recommend this type of fertilizer in order to grow plants with special ornamental characteristics (tall stems, numerous and qualitative flowers that are evenly disposed).

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Influence of controlled release fertilizers on Lilium regale species growth and flowering

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