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THE EFFECT OF IRON NANO-CHELATE AND CYCOCEL ON SOME MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS, PROLIFERATION AND ENHANCING THE QUALITY OF *EUPHORBIA PULCHERRIMA* WILLD

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Abstract

The successful application of various Nano-platforms in medicine under in vitro conditions has generated some interest in agro-nanotechnology. This technology holds the promise of controlled release of agrochemicals and site targeted delivery to improve efficient nutrient utilization and enhanced plant growth. Nano-encapsulation shows the benefit of more efficient use and safer handling of pesticides with less exposure to the environment. Thus, nano-fertilizers can be substituted for conventional fertilizers. Growth reduction in some ornamental plants enhance their overall quality. Cycocel is one of the most important growth retardants which inhibits gibberellin. These experiments were carried out based on a randomized complete block design in three replications to evaluate the effect of various levels of nano iron chelated fertilizer and cycocel on growth parameters of Euphorbia pulcherrima. Treatments were Fe chelated fertilizer (0, 0.9, 1.8, 3.6 and 4.5 g l^{-1}) and Cycocel (0, 500, 1000, 1500 and 3000 mg l^{-1}). Plant height, internode length, shoot number, node number, root length, root number, the length of longest root, the length of shortest root, root volume, fresh and dry weight of root and shoot, leaf number, leaf surface, leaf chlorophyll content, measurement of iron in leaf and the number and permanent of bracts were evaluated. The least plant height and the most shoot, leaf number, root length, root volume, the number and permanent of color bracts were obtained in treatments of 1.8 g l^{-1} iron nano-fertilizer without or with the concentration of 1000 mg l^{-1} cycocel. The most leaf surface, which is a good trait, was calculated in plants treated with 1.8 g l^{-1} iron nano-fertilizer without Cycocel. In some traits such as root volume and chlorophyll content, the minimum amount was seen in the maximum of iron nano-fertilizer and cycocel concentrations. Suitable root characters were severely reduced under the effect of 3000 mg l^{-1} cycocel. Overall, the most suitable treatment, especially for reduction of plant height and enhancing some vegetative traits (such as leaf number) and flowering (such as permanent of bract) is introduced in treatment of 1.8 g l^{-1} iron nano-fertilizer along with 1000 mg l^{-1} Cycocel.

Key words: *Plant growth regulators, Flowering, Soilless culture, Fertilization*

INTRODUCTION

Poinsettia plant of the euphorbia family (*Euphorbiaceae*) as potted and cut flower has great importance in ornamental plant industry. *Euphorbia* family plants have specific primary inflorescence called cyathium. Each cyathium inflorescence usually is surrounded by involucres consists of five continuous leaf origin bracts and the structure of flower is simple (Ghahraman, 1993).

One of the factors affecting the quality of potted and cut flowers is plant nutrition management.

Access to availability, cost and efficiency domestic fertilizer resources using new technology which while more economical to produce, leading to reduce loss of fertilizer and reduce environmental pollution can be very important.

Nanotechnology is opened new opportunities to enhance nutrient use efficiency and minimize the cost of environmental protection (Naderi and Danesh S., 2011). Also with the help of this technology, it is possible to enable the production of higher value-added products and remove environmental toxicity (Garda-Torredey et al., 2002).

The benefits of nano-fertilizers and their superiority over conventional fertilizers can include: increased nutrient uptake with the properties to slow release of the elements; cost reduction; prevent accumulation of high concentrations of soluble salts in the root environment and prevent damage to the plant; reduce the convert of the usable form to unwanted or unusable items in response to usual reaction in the soil; reduce losses due to leaching of nutrients in the root environment and subsequently reduce environmental pollution.

Iron role in the activity of some enzymes such as catalase, peroxidase and cytochrome oxidase has been shown (Blakrishman, 2000). Iron as a cofactor involved in the structure of many antioxidant enzymes and results indicate that in the lack of micro-nutrients elements, antioxidant enzyme activity decreased and therefore the sensitivity of plants to environmental stresses will increase.

The use of chelated iron compounds is the best solution to remove this problem. Iron chelated nano-fertilizer is a unique complex which has 9% of the water-soluble iron.

Some regulators such as cycocel, paclobutrazol, bayleton and daminozide reduce the growth.

Today, a variety of organic and chemical compounds that are artificially made and delay plant growth are used in agriculture (particularly in horticulture and ornamentals industry).

Some ornamental plants, if have less height, are looking more appealing and its transportation is easy.

The effect of plant growth retardants depend on the time and method of application, concentration and species and varieties type, type of target organ and environmental and physiological conditions (James et al., 1999; Latimer et al., 2001).

Plant growth retardants delay cell division and elongation of shoot and restricting the construction of gibberellin reduces internodes length and vegetative growth (Magnitskiy et al., 2006).

Plant retardants inhibit gibberellin biosynthesis. Among these compounds, the most used are mercury chloride and cycocel.

Plant growth retardants, especially cycocel reduced the height of some ornamental plants and increase their quantity and quality characteristics have been used by some researchers (Garner, 2004; Karlovic et al., 2004; Rossini Pinto et al., 2005; Leclerc et al., 2006; Hashemabadi and Zarchini, 2010; Gholampour et al., 2012; Fathi et al., 2012).

Poinsettia plants are including ornamental plants that studies on the effect of nano-fertilizers have been not done on it.

Therefore, the aim of the present study was to increase the quantity and quality of ornamental poinsettia plant using iron nano-chelated and cycocel. If proves appropriate, they can replace with conventional fertilizers and can be reduced contamination of soil, water and environment.

MATERIALS AND METHODS

Cuttings with a height of 15 to 20 cm, each with 3 nodes were prepared from mother plant of poinsettia.

Cuttings due to latex content were placed in water for 24 hours. After this period, cuttings were planted in perlite for rooting. After rooting (60-65 days), cuttings were transferred into substrates.

A number of pots were prepared and cocopeat, municipal compost and soil (1:1:1) were added insides them.

Dimensions of pots were 12 × 12 × 10 cm and medium size was 2 kg. Poinsettia cuttings as the plant samples were grown in pots. Different ratios of iron nano-chelated fertilizers as well as the first experiment factor and different concentrations of cycocel as the second factor were prepared.

EDTA-based iron nano-chelate intake as spray on plants at the beginning of the trial and 30 days later, also using cycocel 30 days after the starting experiment was conducted as spraying.

Levels of experimental factors were: concentrations of 0, 500, 1000, 1500 and 3000 mg/l cycocel and concentrations of 0, 0.9, 1.8, 3.6 and 4.5 g/l of Fe nano-chelated fertilizers (Table 1).

Studies were conducted in a factorial experiment based on randomized complete block design (RCBD) in three replications. Experiment had 75 blocks and three pots were used at each block and in total, 225 pots were used.

Plots were pots containing iron nano-chelated fertilizers and cycocel. Plants were kept in the greenhouse with a temperature of 22-24° C, humidity 85% and were grown in natural light.

Plant height, internode length, shoot number, node number, root length, root number, the length of longest root, the length of shortest root, root volume, fresh and dry weight of root and shoot, leaf number, leaf surface, leaf chlorophyll content, measurement of iron in leaf and the number and permanent of bracts were evaluated.

To measure the length of internodes, in each sample, the length of the lower third of the internodes were calculated.

Shortest and longest root was reported in two ways: among all plants (all observations), shortest and longest root length was measured; the shortest and longest root of three replicates per treatment were measured and divided by 3.

To measure the root volume, at first, the roots were removed from soil and were washed thoroughly under running water.

In a graduated cylinder, poured some water in a particular volume and plant roots to the collar was placed inside the container.

Difference between the measured volumes of water represents the root volume.

In order to measure the dry and fresh weight of plant samples, they quickly were weighed after harvest on a digital scale (fresh weight). Then, the samples were dried in an oven with the temperature of 103°C for 24 h and reweighed (dry weight).

Graph paper was used to measure leaf area. Each leaf was placed on a sheet of paper and it's around was marked with a pen. Area of a square was measured in paper and was multiplied by the number of squares occupied by leaves. The resulting number is the leaf area. Leaf area of each iteration was obtained from the average area of 10 leaves.

For the measurement of chlorophyll a, chlorophyll b and total chlorophyll, 0.5 g leaves was placed in a mortar and after adding 20 ml of acetone 80%, it was absolutely beaten. Green extract was filtered with a funnel and filter paper into 50 ml graduated cylinder. The remaining residue on the funnel was returned to the mortar and 15 ml of acetone 80% were added and beaten. The obtained extract was filtered again. And the remaining residue twice with 5 and 10 ml of acetone 80%, were beaten and filtered, respectively. Finally, acetone 80% extract was brought to a volume of 50 ml. Solutions obtained by spectrophotometer at wavelength (A) 660 and 642.5 nm were read.

Pigments concentration were calculated in ml/g fresh weight.

To measure iron, 0.3 g of dried herb in a temperature of 75°C for 24 h were spilled in 3 ml of mixed acid (100 ml sulfuric acid + 6 g of salicylic acid and 18 ml distilled water) and digestion was carried out on the heater. After bleaching the samples, samples obtained using filter paper was filtered and the volume was brought to 50 ml with distilled water.

Iron content of the samples was obtained using atomic absorption device. Measurements were performed at 120 days after treatment.

Data analysis was carried out using MSTAT-C software and EXCEL software was used to draw graphs.

The mean comparison was carried out using LSD test.

RESULTS AND DISCUSSIONS

The minimum plant height (29.80 cm) and the most shoot (6.00), leaf number (49.23), root length (23.57 cm), root volume (2.23 ml), the number (9.15) and permanent of color bracts (62.33 days) were obtained in treatments of 1.8 g l^{-1} iron nano-fertilizer without or with the concentration of 1000 mg l^{-1} cycocel.

Maximum plant height (49.46 cm) and internode length (4.33 cm) were calculated in plants treated with 4.5 g l^{-1} iron nano-fertilizer without cycocel.

There was no any positive effect between increasing internode length and increasing iron nano-fertilizer concentration.

The minimum root volume (1.26 ml) and chlorophyll content (2.19 mg g^{-1} F.W.) were obtained in plants treated with maximum concentrations of iron nano-fertilizer and cycocel (4.5 g l^{-1} and 3000 mg l^{-1}).

Maximum chlorophyll content (6.61 mg g^{-1} F.W.) was obtained in plants treated with 3.6 g l^{-1} iron nano-fertilizer with the concentration of 500 mg l^{-1} cycocel.

Less shoot number (2.66) was obtained in plants treated with 4.5 g l^{-1} iron nano-fertilizer without cycocel.

Maximum (30.30) and minimum (18.30) node number were seen in plants treated with control and 1.8 g l^{-1} iron nano-fertilizer along with the concentration of 500 mg l^{-1} cycocel.

The largest (9.46) and smallest (6.63) number of root were observed in plants treated with 3.6 g l^{-1} iron nano-fertilizer without cycocel and 0.9 g l^{-1} iron nano-fertilizer along with the concentration of 3000 mg l^{-1} cycocel, respectively.

The biggest leaf surface (19.55 cm^2), which is a good trait, was calculated in plants treated with 1.8 g l^{-1} iron nano-fertilizer without cycocel.

The least leaf surface (9.79 cm^2) was calculated in plants treated with 4.5 g l^{-1} iron nano-fertilizer along with 1500 mg l^{-1} cycocel.

In some traits such as root volume and chlorophyll content, the minimum amount was seen in the maximum of iron nano-fertilizer and cycocel concentrations. S

uitable root characters were severely reduced under the effect of 3000 mg l^{-1} cycocel.

Maximum (112.40 mg/1000 ml) and minimum (41.50 mg/1000 ml) concentration of iron were seen in plants treated with 500 mg l^{-1} cycocel without iron nano-fertilizer and 0.9 g l^{-1} iron nano-fertilizer along with 3000 mg l^{-1} cycocel, respectively.

Overall, the most suitable treatment, especially for reduction of plant height and enhancing some vegetative traits (such as leaf number) and flowering (such as permanent of bract) is introduced in treatment of 1.8 g l^{-1} iron nano-fertilizer along with 1000 mg l^{-1} cycocel.

The results of this study indicated the role of iron nano-chelated fertilizer and cycocel to change some vegetative growth, flowering and physiological indexes of poinsettia plant, so that the change in the indexes of plant height, internode length, number and area of leaves, number and length of roots, dry/fresh weight of foliage shoot and root, number of bracts and iron and chlorophyll contents were significant.

Given the new nanotechnology and the growing trend of studies in the technology, there are not many reports about the effect of the fertilizer on plant growth and development.

Similar results were obtained in several plants (Abbas et al., 2009; Sheykhbaglou et al, 2010). Peivandi et al. (2011) studies on comparing the effect of iron nano-chelated fertilizer on *Ocimum basilicum* showed that replacing iron fertilizer produced with nanotechnology compared with conventional iron fertilizer at the appropriate concentration or less increase the growing quantity and quality of this species.

These researches showed that differences in the mean of shoots fresh weight, shoot dry weight, dry/fresh weight of leaves and roots in the different treatments was significant.

The highest mean shoot fresh weight (5.84 g), root fresh weight (0.56g), fresh weight of leaf (2.70 g), shoots dry weight (0.71 g), root dry weight (0.13 g), leaf dry weight (0.3 g) and root length (14.41 cm) was observed in the treatment of 1 kg/h of iron nano-chelate.

In the present research, maximum root length obtained at a concentration of 1.8 g l^{-1} that confirms the results obtained by the Peivandi et al. (2011).

There are similar results in relation to the fresh/dry weight of vegetative organs, so that fresh/dry weight of shoots and roots in treatment 4.5 g l^{-1} were maximized in iron nano-chelate.

With increasing concentration of iron nano-chelated fertilizer, the content of chlorophyll a, b and a + b increased compared to control.

The highest chlorophyll content was observed in Fe nano-fertilizer with higher concentrations (5 kg/ha).

The highest amount of leaves chlorophyll in our study was not found in the highest concentration of iron nano-chelate (4.5 g l^{-1}), but was obtained at a concentration of 3.6 g l^{-1}.

Increased iron may be associated with a decrease in chlorophyll content and photosynthetic rate and may lead to reduced growth (Chatterjee et al., 2006).

Cycocel is one of the plant growth regulators in euphorbia (such as poinsettia) and Chrysanthemum (Fathi et al., 2012). Gholampour et al. (2012) study on ornamental cabbage (*Brassica oleracea*) showed that with increasing concentration of cycocel, plant height was reduced. All plants treated with cycocel were shorter than control plants.

These researches showed that 1500 mg l^{-1} cycocel were produced shortest plants (10.79 cm) that was shorter compared to the control plants (15.20 cm).

With increasing cycocel concentration, stem length also declined. Similar results were observed in the present study, so that the maximum height of the stem in the plant was observed and with increased cycocel to 1000 ml l^{-1}, stem length was reduced.

Although the stem height in the plants treated with 1500 and 3000 mg l^{-1} cycocel was a little more than stem height in plants treated with 1000 mg l^{-1} cycocel, was much less the height of the control plants.

Reduced plant height by cycocel application has been observed in many plant species (Oliveira and Browning, 1993; Garner, 2004; Karlovic et al., 2004; Rossini Pinto et al., 2005; Hashemabadi and Zarchini, 2010). Karlovic et al (2004) reported on reduced height in chrysanthemum (*Chrysanthemum* L. cv. Revert) after application of 2000, 3000 and 4000 mg l^{-1} cycocel. Hashemabady and Zarchini (2010) showed that minimum plant height (29.93 cm) obtained in rose using 1500 mg l^{-1} cycocel. Shoot length in control plants was 35.70 cm. The researchers found a significant reduction in the length of stem after application of cycocel.

This study confirms other research studies. The present study shows that cycocel play effective role on changing fresh/dry weight on the ground and underground part, as well as leave iron and chlorophyll. Gholampour et al. (2012) Study on ornamental cabbage (*Brassica oleracea*) showed that the maximum chlorophyll index with 1000 mg l^{-1} cycocel were obtained after 60 days (18.50) and at a concentration of 1500 mg l^{-1} after 90 days (20.14).

Plant growth retardants increase the content of cytokinin which leads to increased levels of leaf chlorophyll (Rossini Pinto et al., 2005). Our study revealed that the highest and lowest chlorophyll in the leaves, was in the plants treated with the lowest (500 mg l^{-1}) and highest (3000 mg l^{-1}) concentrations of cycocel, respectively.

CONCLUSIONS

The most suitable treatment, especially for reduction of plant height and enhancing some vegetative traits (such as leaf number) and flowering (such as permanent of bract) is introduced in treatment of 1.8 g l^{-1} iron nano-fertilizer along with 1000 mg l^{-1} cycocel.

Maximum root length was obtained at the same concentration of 1.8 g l^{-1}.

Cycocel play effective role on changing fresh/dry weight on the ground and underground part, as well as leave iron and chlorophyll.

The highest chlorophyll content was observed in Fe nano-fertilizer with higher concentrations (5 kg/ha). The highest and lowest chlorophyll in the leaves, was in the plants treated with the lowest (500 mg l^{-1}) and highest (3000 mg l^{-1}) concentrations of cycocel, respectively.

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