

CREATING OPTIMAL NUTRIENT CONDITIONS IN SOIL AND PLANTS DURING THE FLOWER INDUCTION PROCESS OF SWEET LIME (*Citrus limettioides*) UNDER CALCAREOUS SOIL CONDITIONS TO INCREASE THE YIELD

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ABSTRACT

A study was conducted on 6-year-old sweet lime trees on the Mexican lime (*Citrus aurantifolia* Swingle) rootstock in a randomized complete block design with ten treatments and three replications in southern Fars to determine the appropriate time of plant supplementary feeding to increase flower production and the yield in calcareous soils. In 2016 and 2017, 40 trees that had the same planting and growing conditions, were selected to determine the flower induction time. Ten times (22nd of October, 1st, 11th, and 20th November, December, and January), four branches that had a length of 120 cm and a diameter of half a centimeter were selected on each tree in four geographical directions. At each time, the branches of three trees were ringed at a distance of 120 cm from the tip of the branch, and all the leaves were removed. At the time of flower emergence, the number of flowers was counted. The results showed that the flower induction in these trees was in early December. Based on the results of this experiment and previous research in this field, in September 2017, the 7-year-old trees grafted on the Mexican lime rootstock were treated with the foliar application of nitrogen as urea [CO(NH₂)₂] and zinc as zinc sulfate (ZnSO₄) at three concentrations including 0, 3, and 5 g·L⁻¹, alone and by combination in two separate pieces in a randomized complete-block factorial design including nine treatments and three replications to increase the yield using elements involved in the flowering. At the harvest time, fruit juice, TSS, TA, vitamin C, average fruit weight, and single tree yield were measured. According to the results, the combination of urea and zinc sulfate at a concentration of 5 g·L⁻¹ led to an increase in the average fruit weight (58 g) and the yield (68 kg) compared to the control.

Key words: calcareous soil, flower induction, girdling, Mexican lime, urea, zinc sulfate

INTRODUCTION

As a result of drought in recent years, disruptions have occurred in the water sources of the agricultural sector. The importance of proper use of available water resources has doubled [Thomas et al. 2015]. Perhaps

the only way is proper management and proper utilization of available water, the maximum increase in yield and quality fruit production without increasing the area under fruit trees, especially citrus [Aboutalebi

and Hassanzadeh 2013]. Flower formation is the prelude to fruit production. Identifying the effective factors in flower formation will play an important role in the production of the commercial product [Hosseini et al. 2021]. From a physiological point of view, flowering of fruit trees is affected by various internal factors such as seedlings, the relative amounts of carbohydrates and nitrogen, hormones, and yield [Iglesias et al. 2007].

The flower formation in citrus in subtropical regions in terms of winter cold occurs once a year, but in tropical regions throughout the year or in other words several times. In subtropical regions, the flower induction occurs with stunted growth during winter rest and low temperatures (less than 20 to 25°C) for more than 300 hours or a dry period of more than 30 days [Micheloud et al. 2018]. After the seedling period and carbohydrate storage, when the average air temperature is less than 10°C, and with hormonal imbalance in the leaves, activation and joining of the internal flowering mechanisms and the presence of high GA₃ in the leaves, the flower induction is transported from the leaves to the lateral vegetative buds to become reproductive buds. The presence of the leaf has been proven as the first factor for carbohydrate production and the flower induction site in citrus. Removing leaves because of winter cold, the salinity of water and soil and/or strong winds reduce the flowering in spring. This decrease in the number of flowers is the result of not expressing the flowering gene. In citrus, the presence of leaves is most likely to be essential for the flowering [Nishikawa et al. 2013].

The number of flowers and fruit formation also depends on the nutritional conditions of the tree. At the time of flowering and fruit formation, the elements nitrogen, phosphorus, and potassium in the old leaves are severely reduced but have caused a sharp increase in these elements in the new leaves and fruits [Sulistiawati et al. 2017]. Girdling causes carbohydrates to accumulate above the girdled branch [Asao and Ryan 2015]. This phenomenon is indirectly a sign of the involvement of carbohydrates in flowering, which in turn causes the carbohydrate factor to cause flowering [Asao and Ryan 2015]. The role of carbohydrates and their support in flower induction is known that this action increases the induction of flower buds

by girdling [Gawankar et al. 2019]. In many herbaceous plants, the leaf understands the need for cooling and the light cycle, or both to begin flowering [Nishikawa et al. 2013]. The most important factors that affect the flowering are ambient temperature, water, and nutrients, as well as carbohydrates and hormones. The number of the appeared flowers depends on the age of the tree, cultivar, and other environmental and managerial conditions [Sulistiawati et al. 2017]. Internal and external factors are involved in the rate of flower fall in the plant. Nitrogen deficiency during flowering is one of the causes of severe flower fall and reduced fruit formation and thus reduced yield [Hosseini et al. 2021]. Foliar application of urea for four consecutive years during the winter has significantly increased the number of flowers and yield in Valencia oranges [Abdel-Aziz and El-Azazy 2016].

Nitrogen is a key component in mineral fertilizers used in citrus trees; this effect is increasingly evident in the growth, appearance, and production of quality fruit than any other element [Thomas et al. 2008]. The foliar application of nitrogen in the form of urea is one of the most critical methods of citrus fertilization in Florida and other citrus growing regions of the world because this method reduces nitrate leaching and its entry into groundwater [Bondada et al. 2001]. Researchers have reported that nitrogen in the form of urea through the foliar application in the supplemental diet is four times more effective than the soil application, with increased yields, improved fruit color, control of vegetative growth, and reduced leaching as the most significant effects [Salem et al. 2004].

Zinc is a vital micronutrient in flower formation and fruit production. The importance of zinc in photosynthesis is related to the presence in a part of the carbonic anhydrase enzyme in all photosynthetic tissues of the plant, which is required for chlorophyll synthesis. Zinc is equally valuable in the production of auxin by participating in the production of the amino acid tryptophan in plants [Casanova-Sáez et al. 2021]. Zinc performs a prominent role in the regulatory structure of cofactors, various enzymes, and proteins. Furthermore, at the level of plant organs, the pivotal and valuable role of this element is considered as a key component of the structure of gene transcription regulation [Broadley et al. 2007]. The most important importance

of zinc is due to its role in the formation of thousands of proteins in plants [Asadi-Kangarshahi et al. 2005]. The beneficial effects of zinc sulfate on increasing the yield and quality of Kinnow tangerine [Razzaq et al. 2013] and sweet limes [Behrooznam and Hassanpour 2005] have been reported.

Recognizing the effective time in inducing the flowering as well as the process of forming a complete, suitable, and quality flower, is critically important and can help gardeners to eliminate the deficiencies of effective elements in flowering during the time before inducing the flowering through soil tests and plant leaf analysis. Moreover, considering the time of absorption of elements inside the plant through soil or the foliar application, it provides optimal conditions for the plant during the flower induction process so that the plant does not have any restrictions in terms of lack of necessary elements and their absorption. Creating optimal nutritional conditions in the plant and soil during the flower induction process can be one of the factors in achieving a high yield in the sweet lime plant.

Materials and methods

Plant materials and project location. The experiment to determine the flower induction time was carried out in the citrus research garden of Islamic Azad University, Jahrom Branch, with geographical coordinates of longitude 53°33' and latitude 28°30' at an altitude of 1,179 meters above sea level in 2016 and 2017. The soil available in the study area was loam-silt soil texture, with total lime 46% and activated lime less than 10%. In each year, forty 5- to 6-year-old sweet lime trees that possessed the identical conditions in terms of growth, irrigation, and nutrition, were selected.

Determine the flower induction time. The ringing operations were performed on 22nd of October, 1st, 11th, and 20th November, December, and January to determine the flower induction time. The branches of this year that had a length of 120 cm and a diameter of a half a centimeter was selected on each tree in four geographical directions. They were ringed at a distance of 120 cm from the tip of the branch, and all the leaves above the ringing site were removed. This experiment was performed as a randomized complete-block design with ten treatments (time) and each treatment consisted of three replications (three trees)

and each replication consisted of four branches from four directions of the plant. In each time period, one tree was considered as a control. With the onset of the flower induction in February and March of each year, all branches of the treated trees were inspected, and the flowers were counted and the initial inventory was performed carefully.

Applying the nutritional treatments. After obtaining the results, using data analysis for two consecutive years, the time range of onset of flower induction and reaching the maximum flowering was determined. A period of one month before the first date of onset of flower induction was considered to ensure the flower induction time period and to be able to reduce the deficiencies of the required elements and involved in the flowering before the beginning of flowering with a better opportunity.

In 2017, at the appropriate time, soil tests [Thomas et al. 2015] and plant leaf analysis [Falivene 2016] was performed to eliminate the possible deficiencies of effective elements in flowering in the soil and inside the plant. By characterizing the results of soil and leaf analysis as well as the flower induction test and being inspired by the results of other researchers' research on the role of elements involved in flowering and increasing the yield, zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) 21% (Eurosolids, Netherlands) and urea [$\text{CO}(\text{NH}_2)_2$] 46% (Agricultural Support Services Co., Shiraz) were prepared using nitrogen and zinc elements and the foliar application according to the concentration of treatments (0, 3, and 5 $\text{g}\cdot\text{L}^{-1}$ individually and by combination) was performed on the target trees before the tree entered the flower induction stage. This experiment consisted of nine treatments and three replications and each replication included a sweet lime tree (27 trees), which was carried out on a total of 54 seven-year-old sweet lime trees on the Mexican lime rootstock in a randomized complete-block factorial design in two separate gardens with a pressurized drip-irrigation system under the same management conditions (irrigation, nutrition, soil texture and tree age).

At the beginning of October of the following year and with the beginning of harvest, yield, the average weight of single fruit, total soluble solids (TSS), total acid (TA), vitamin C, fruit juice percentage, and firmness of fruit tissue of both gardens were measured.

An ocular refractometer (MT-098, Singapore) was used to measure soluble solids. The titration method with NaOH 0.1N was used using phenolphthalein reagent to determine the amount of TA [Andrews 2002]. L-Ascorbate or vitamin C was measured at 515 nm by a spectrophotometer (HALO×B-10, Dynamica) [Bor et al. 2006]. A fruit firmness tester (penetrometer) (FTO11 (0-11Lbs, China) with probe No. 8 was used to measure the firmness of fruit tissue. The average weight of each fruit was measured using a digital scale with an accuracy of 0.01 g. A 20 cm caliper (Shoka Golf) with an accuracy of 0.001 was used. To measure the amount of fruit extract, 15 fruits were randomly harvested from each replication and each of them was weighed with a digital scale and their extract was obtained with a manual juicer and weighed again with a digital scale. By calculating the ratio of the weight of the extract to the initial weight of the fruit and multiplying the number obtained by 100, the percentage of the fruit extract was calculated.

Statistical analysis. The data were statistically analyzed using the SAS 9.1 software in a randomized complete-block design and the mean data were compared through Fisher's PLSD test and the Excel software was used to plot the graphs. The experiment was performed in two separate gardens under the same management conditions. After the initial combined analysis, since the effect of location on all studied traits was not significant, data analysis was performed based on the mean data of the two locations.

RESULTS

Determine the flower induction time. Based on the results of the analysis of variance, ringing and defoliation time possessed a significant effect on the number of flowers at a $p < 0.01$. Although the effect of the year in this experiment alone was not significant, the interaction of the time of applying the treatment and the year was significant at a 1% level (Tab. 1).

The results of comparing the means in relation to comparing the effect of time of applying the girdling and defoliation treatment on the number of flowers indicated that the highest rate of flowering in both years was related to the control treatment, in which the flower induction process took place naturally (Fig. 1).

Furthermore, the analysis of variance of the data in relation to the effect of girdling and defoliation time on the number of flowers showed that in both experimental years, the treated trees failed to produce flowers before November (Fig. 2A, 2C), which means that until then all factors that affect the flower induction, have not been completed (Fig. 1).

A comparison of the interaction of the year and time of application of girdling and defoliation treatments on the number of flowers showed that the highest flower production in 2016 (1082) and 2017 (1376) was related to the control treatment, which showed a significant difference with all treatments, followed by the treatment of 31 December 2017 (755 flowers) (Fig. 2B, 2D) and the treatment of 20 January 2017 (600 flowers), but no significant difference was observed between them (Fig. 1).

A comparison of the effect of time of applying girdling and defoliation treatments on the number of flowers per year showed that in 2016, with the application of treatments at different times, the flower production process increased, which can be considered normal, while in 2017, this trend has changed. Considering the weather situation in 2017 in the tested area and the lack of proper fall and winter rainfall and lack of humidity and severe temperature differences between night and day (unfavorable climatic conditions) have not been ineffective in the occurrence of this difference (Fig. 1).

Meteorological statistics registered on the meteorological site [Meteorological statistics 2017] in relation to the years 2016 and 2017 related to the months of September, October and November in the region showed that the average humidity in the fall of 2016 was more than 55% and the amount of rainfall was more than 170 mm, while the total amount of rainfall in the mentioned months in 2017 has been less than 40 mm. Furthermore, the temperature difference between night and day for the announced period has been reported more than in 2016 (Fig. 3).

According to the results of the analysis of variance of the data in the experiment of foliar application, urea showed a significant effect on all studied traits. The effect of zinc sulfate on all traits except the percentage of fruit juice was also significant. In all traits, a significant interaction was observed between urea and zinc

Table 1. Analysis of variance in respect of the effect of girdling and defoliation time on the flower number

Source of variation	Degrees of freedom	Sum of square	Mean of square	F value
Year (Y)	1	4.0	4.0	0.40ns
Error Y	6	60.0	10.0	–
Treating time (T)	10	11690.0	1169.0	228.9**
Y × T	10	222.5	22.2	4.36**
Error	60	306.4	5.1	–
Total	87	12282.9	–	C.V. = 18.3%

^{ns}not significant, ^{**}significant at $p < 0.01$

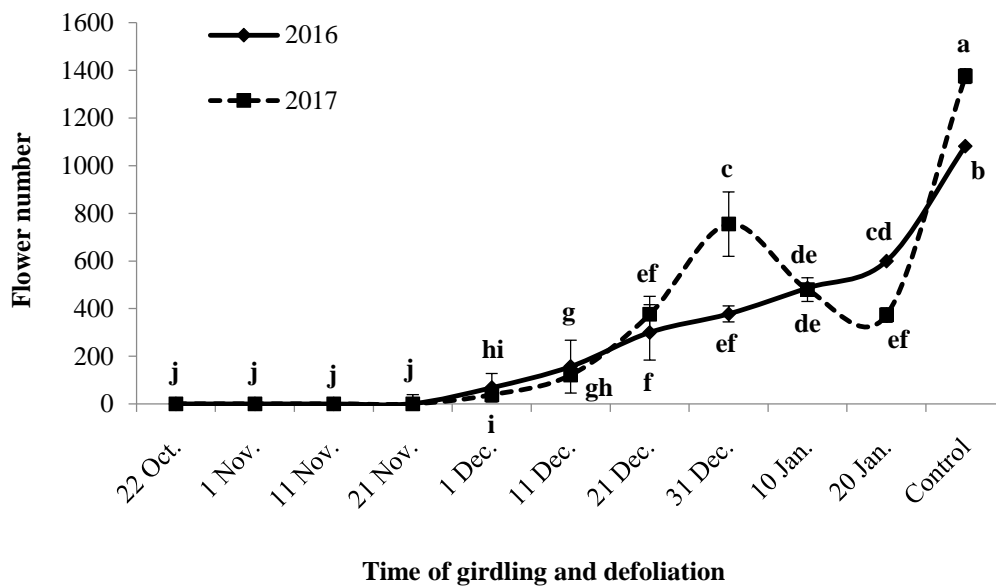


Fig. 1. Effect of girdling and defoliation time on the flower number in two successive years. The letters showed significant difference according to PLSD test ($p < 0.05$)



Fig. 2. Defoliation and girdling in 22 October (A) and 31 December (C); appearance of new flowers in spring in relation to defoliation in 22 October (B) and 31 December (D)

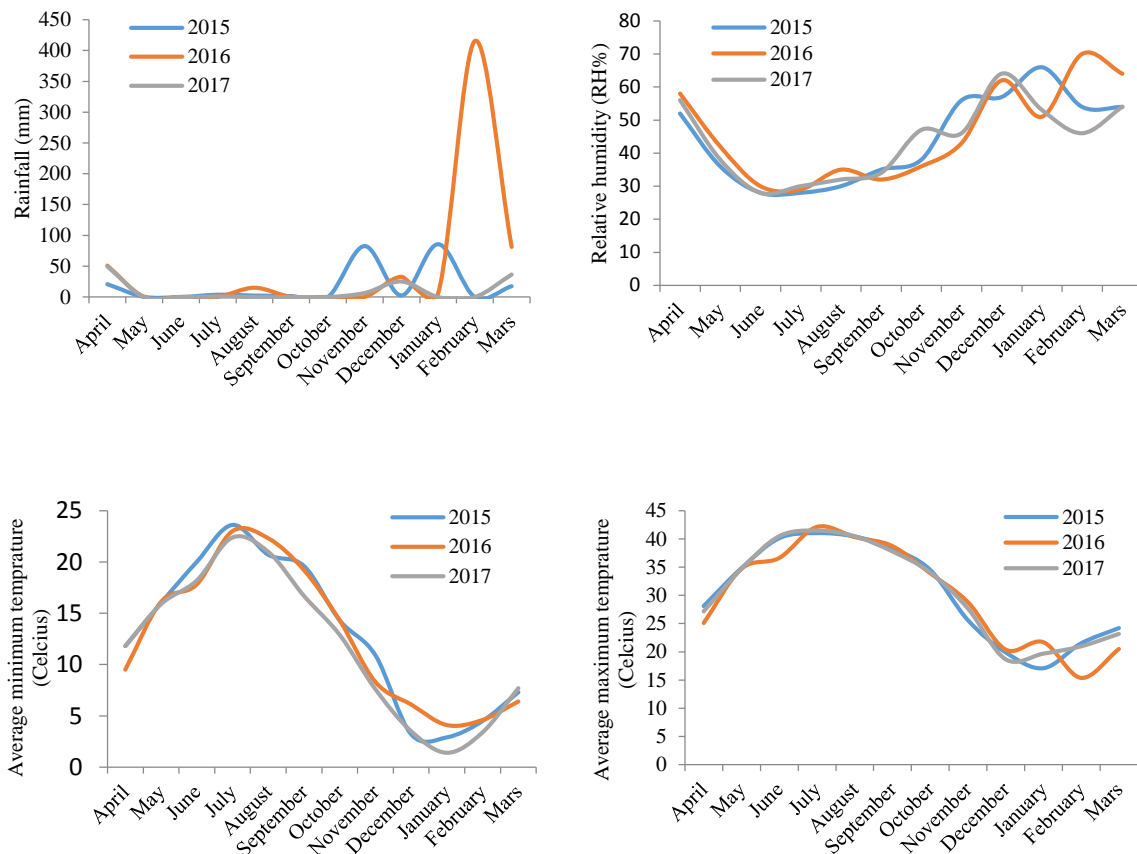


Fig. 3. Monthly meteorological statistics including rainfall, relative humidity, and the minimum and maximum temperature of three successive years (2016–2017)

Table 2. Analysis of variance in relation to the effect of different amounts of urea and zinc sulfate on quantitative and qualitative attributes of Sweet lime fruits

Source of variation	Degrees of freedom	Mean squares						
		Total soluble solid	Titration acid	Vitamin C	Average fruit weight	Fruit juice	Fruit firmness	Single tree yield
Replication	2	1.08 [*]	0.41 ^{ns}	2.9 ^{ns}	7.1 ^{ns}	2.9 ^{ns}	0.06 ^{ns}	264.2 ^{ns}
Urea (N)	2	1.77 ^{**}	22.48 [*]	176.4 ^{**}	3642.4 ^{**}	61.2 ^{**}	5.68 ^{**}	5335.6 ^{**}
Zinc sulfate (Zn)	2	0.89 [*]	5.88 ^{**}	17.5 ^{**}	1285.1 ^{**}	8.4 ^{ns}	0.45 ^{**}	4450.1 ^{**}
N × Zn	4	1.57 ^{**}	17.18 ^{**}	42.7 ^{**}	1225.2 ^{**}	13.8 ^{**}	1.78 ^{**}	3060.4 ^{**}
Error	16	0.20	0.18	2.0	78.4	2.6	0.04	208.6
C.V.%		4.8	2.7	3.6	6.6	4.0	1.8	8.3

ns, * and ** non-significant, significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

sulfate (N × Zn) (Tab. 2). Based on this, the comparison of the interaction of the two factors was discussed.

Comparison of the interaction effect of urea and zinc sulfate on the studied traits

Percentage of total soluble solids (TSS). The most elevated level of TSS in the treatment of urea (3 g·L⁻¹) or a combination of urea (3 g·L⁻¹) and zinc sulfate (5 g·L⁻¹) was observed (10.35 and 10.05%, respectively) and the lowest was for the control treatment (8.40%). With increasing urea concentration from 0 to 3 g·L⁻¹, in the absence of zinc sulfate, the amount of TSS increased significantly. This change in the presence of 3 and 5 g·L⁻¹ of zinc sulfate decreased and increased significantly, respectively. With increasing urea concentration from 3 to 5 g·L⁻¹, TSS decreased significantly in the presence of 5 g·L⁻¹ of zinc sulfate or not consuming zinc sulfate. This trend was significantly reduced in the presence of 3 g·L⁻¹ of sulfate (Tab. 3).

Total acid (TA). The most extraordinary amount of TA was observed for the treatment of 5 g·L⁻¹ of zinc sulfate alone (0.198 mg) and the lowest for the control treatment (0.130 mg). With increasing urea concentration from 0 to 3 g·L⁻¹, in the absence of zinc sulfate, the amount of TA increased significantly. This change in the presence of 3 and 5 g·L⁻¹ of zinc sulfate was an insignificant increase and a significant decrease, respectively. By increasing the urea concentration from 3 to 5 g·L⁻¹, TA decreased significantly in the presence or absence of zinc sulfate (Tab. 3).

Vitamin C. The most elevated vitamin C content was observed for the treatment of 3 g·L⁻¹ of urea alone (47.15 mg) and the lowest for the treatment of 3 g·L⁻¹ of zinc sulfate alone (31.75 mg). With increasing the concentration of urea from 0 to 3 g·L⁻¹, in the absence or presence of zinc sulfate, the vitamin C content increased significantly. By increasing the urea concentration from 3 to 5 g·L⁻¹, the vitamin C content in all levels of zinc sulfate decreased, which was not significant at a concentration of 5 g·L⁻¹ of zinc sulfate (Tab. 3).

Average fruit weight. The highest average fruit weight was observed for the combined treatment of urea and zinc sulfate at a concentration of 5 g·L⁻¹ (189 g) and the lowest for the treatment of 3 g·L⁻¹ of zinc sulfate alone (105 g). With increasing urea concentration from 0 to 3 g·L⁻¹, at all levels of zinc sulfate, the average weight of the fruit did not change significantly. By increasing the urea concentration from 3 to 5 g·L⁻¹, the average fruit weight in the presence of 3 and 5 g·L⁻¹ of zinc sulfate demonstrated a significant increase, while in the absence of zinc sulfate, the average fruit weight decreased insignificantly (Tab. 3).

Percentage of fruit juice. The most significant percentage of fruit juice was related to the treatment of 3 g·L⁻¹ of urea alone as well as to the combined treatment of 5 g·L⁻¹ of urea and 3 g·L⁻¹ of zinc sulfate (43.6% and 43.2%, respectively) and the lowest was observed for the treatment of 5 g·L⁻¹ of zinc sulfate alone (35 %). By increasing the urea concentration from 0 to 3 g·L⁻¹, in all levels of zinc sulfate, the per-

Table 3. Interaction of urea and zinc sulfate on the quantitative and qualitative attributes of sweet lime fruit

Characteristics Urea × Zinc sulfate		Total soluble solid (%)	Total acid (g/100 ml)	Vitamin C (mg/100 ml)	Average fruit weight (g)	Fruit juice (%)	Fruit firmness (kg/cm ²)	Single tree yield (kg)
Urea 0	Zinc sulfate 0	8.40 ^c	0.13 ^f	39.60 ^c	131 ^c	39.0 ^c	7.37 ^b	175 ^c
	Zinc sulfate 3	9.07 ^{bc}	0.157 ^d	31.75 ^e	105 ^e	37.7 ^{cd}	5.82 ^{cde}	131 ^e
	Zinc sulfate 5	9.65 ^{ab}	0.198 ^a	33.87 ^{de}	126 ^{cd}	35.0 ^d	7.93 ^a	130 ^e
Urea 3	Zinc sulfate 0	10.35 ^a	0.181 ^b	47.15 ^a	127 ^{cd}	43.6 ^a	5.72 ^{def}	210 ^b
	Zinc sulfate 3	8.60 ^c	0.162 ^{cd}	42.32 ^b	115 ^{de}	42.2 ^{ab}	5.87 ^{cd}	164 ^{cd}
	Zinc sulfate 5	10.05 ^a	0.164 ^c	42.25 ^b	125 ^{cd}	40.2 ^{bc}	5.25 ^g	184 ^c
Urea 5	Zinc sulfate 0	9.10 ^{bc}	0.148 ^e	35.48 ^d	126 ^{cd}	39.0 ^c	5.67 ^{ef}	176 ^c
	Zinc sulfate 3	8.78 ^c	0.136 ^f	39.82 ^c	154 ^b	43.2 ^a	5.92 ^c	148 ^{de}
	Zinc sulfate 5	8.55 ^c	0.136 ^f	41.60 ^{bc}	189 ^a	42.2 ^{ab}	5.58 ^f	243 ^a

Means with the same letters in each column are not significantly different according to LSD test at 5% level of probability

centage of fruit juice indicated a significant increase. By increasing the urea concentration from 3 to 5 g·L⁻¹, the percentage of fruit juice in the presence of 3 and 5 g·L⁻¹ of zinc sulfate revealed an insignificant increase, while in the absence of zinc sulfate, the percentage of fruit juice decreased significantly (Tab. 3).

Fruit firmness. The highest fruit firmness was observed for the treatment of 5 g·L⁻¹ of zinc sulfate (7.93 kg/cm²) and the lowest was related to the combined treatment of 3 g·L⁻¹ of urea and 5 g·L⁻¹ of zinc sulfate (5.25 kg/cm²). By increasing the urea concentration from 0 to 3 g·L⁻¹, in the absence of zinc sulfate or the use of 5 g·L⁻¹ of zinc sulfate, fruit firmness decreased significantly. This trend did not change significantly in the presence of 3 g·L⁻¹ of zinc sulfate. With increasing the concentration of urea from 3 to 5 g·L⁻¹, fruit firmness in the absence of zinc sulfate or the use of 3 g·L⁻¹ did not change significantly, but at the level of 5 g·L⁻¹ of zinc sulfate increased significantly (Tab. 3).

Yield. The highest yield was observed for the combined treatment of urea and zinc sulfate at a concentration of 5 g·L⁻¹ (243 kg per tree) and the lowest was related to the treatment of 3 or 5 g·L⁻¹ of zinc sulfate alone (131 and 130 kg per tree, respectively). By increasing the urea concentration from 0 to 3 g·L⁻¹, the yield increased significantly at all levels of zinc sulfate. With increasing urea concentration from 3 to 5 g·L⁻¹, the yield demonstrated a significant increase in the presence of 5 g·L⁻¹ of zinc sulfate, while it decreased in the absence of zinc sulfate or the use of 3 g·L⁻¹ of zinc sulfate (Tab. 3).

DISCUSSION

By determining the time period of the flower induction in the desired plant in an area, it is possible to plan and manage nutrition and irrigation in order that the plant to be able to make optimal use of the important elements involved in flowering before entering the flower induction stage, so that the plant has no restriction in absorbing these elements, leading to an increase in the quantity and quality of induced buds and ultimately increasing the yield of the product. The beginning of the flower induction in both years indicated the application of treatments from the beginning of November onwards. In citrus, the flower induction of buds begins with the cessation of vegetative growth

during plant rest. This period of growth stagnation occurs in subtropical regions because of winter cold (in the range of 12 to 15°C) and in tropical regions because of drought periods (longer than 30 days) [Garcia-Luis and Guardiola 2000].

In citrus like many tropical and subtropical plants, cold temperatures can induce the flowering [Wilkie et al. 2008]. Temperatures above 30°C during the day and above 25°C at night prevent flower bud formation, and low temperatures accelerate the flowering [Distefano et al. 2018]. Each citrus tree produces between 100,000 and 200,000 flowers (depending on cultivar, location, and season), which only about one to two percent of all flowers are turned into fruits [Sulistiawati et al. 2017]. Flower induction, flower initiation and understanding the citrus flowering sequences are important for fruit production because they are the first step in the reproductive process. Citrus trees are evergreen, but when the trees are exposed to severe cold in winter or damage from the wind, water salinity, or severe drought and lose some of their leaves, the number of flowers decreases the following spring. This reduction in the number of flowers is the result of suppressing the induction of flowers by the removed leaves.

Girdling and ringing are the treatments that separate a strip of bark from around the stem or trunk. These treatments have been used for many years in the production of commercial citrus to enhance the flowering. These methods probably suppress the growth of stems and vegetative roots and induce citrus flowering [Nishikawa et al. 2012].

The results of the analysis of variance of comparing the effect of time of applying the girdling and defoliation treatments on the number of flowers showed that the flower induction in the target area began at the beginning of November and continues almost until the end of January. These results were in line with those reported by Wilkie et al. [2008] who stated that in citrus like many tropical and subtropical plants, cold temperatures can induce the flowering.

Based on the results of the analysis of variance, the experiment of the flower induction, the girdling and defoliation time had a significant effect on the number of flowers at a 1% level of LSD test, which showed the highest rate of flowering in both years. Given that the control treatment did not involve any girdling and defoliation operations and no stress was applied to it,

it was predictable that the highest number of flowers during both years of the experiment was related to the control treatment. In both years of experimentation, the treated trees failed to produce flowers before November, and this is because that until then all the effective factors in inducing the flowering had not been completed. The results of this experiment were in line with those reported by Nebauer et al. [2006] who stated that citrus flowering occurs after the seedling period and exposure to low temperatures and the short days of winter. The beginning of the flower induction in both years indicated the application of treatments was from the beginning of November onwards, which was in line with the results reported by Rikande [2013].

The effect of the foliar application of nitrogen on plant nutrition is greater than adding it to the soil. The need for nitrogen in the plant is maximized during the flowering and fruit formation period. Increasing the amount of nitrogen in the flower buds lead to increase leaf area, ovule life period, and duration of pollination and inoculation. As a result, fruit formation is more abundant and is also larger in size, and the yield is increased [Albrigo and Syvertsen 2001].

All plant life processes depend on nitrogen. It plays a vital role in amino acids, proteins, and sugars. The most active nitrogen compounds occur in the protoplasm and nucleus. Enzymes accelerate biological processes. A large level of nitrogenous compounds is required for normal cell division, growth, and respiration, which practically affects the absorption and distribution of all other elements and is very important for the tree during flowering and fruit formation. Its proper function in plant nutrition depends on the proper amount of other essential elements. The results of the foliar application showed that different concentrations of nitrogen alone could improve photosynthesis and therefore increase the level of carbohydrates, TSS, TA, fruit juice percentage, fruit yield. Furthermore, different concentrations of zinc alone increased TSS and TA compared to the control, while the combined treatment of nitrogen and zinc at the most elevated concentration showed a significant increase in yield, average fruit weight, fruit juice percentage, and an insignificant increase in vitamin C, TA, and TSS. The results of this experiment showed that nitrogen was involved in the proper growth of the plant, influencing other elements and combining with zinc sulfate

in activating various enzymes, auxin formation, chlorophyll formation, photosynthetic function, various metabolic activities of the plant, regulating the balance between carbon dioxide and water and carbonic acid, regulating water relations and increasing the improvement of water absorption in plants. Akbari et al. [2007] found that different concentrations of urea increased the number of flowers, stimulated ovary growth, and increased fruit formation in Valencia orange trees on the bitter orange rootstock and observed the most notable effect at a concentration of 1% urea. They also reported the most significant effect on increasing the number of flowers at the time of the foliar application, nine weeks before all flowers.

The results of the effect of amount and method of application of zinc sulfate on the yield and quality of the Unshiu tangerine in the form of surface dispersion and foliar application showed that the highest yield and concentration of zinc per leaf was obtained at a concentration of 4 g·L⁻¹ zinc sulfate, which was 23% higher than the control. The highest yield and zinc concentration were related to the foliar application [Asadi Kangarshahi et al. 2005].

It has been reported that in addition to nitrogen consumption, the time of application also possesses an extensive effect on fruit formation and the reduction in flower and fruit shedding so that the use of 1035 grams of nitrogen per the Washington navel orange in early November had the most beneficial effect [Ruiz et al. 2001]. The foliar application of urea in citrus trees at a concentration of one to two percent during January will increase the flowering period and fruit formation and will also reduce flower shedding [Kumar et al. 2021]. The use of nitrogen in citrus fruits has increased fruit weight, juice content, vitamin C, and yield [Albrigo and Syvertsen 2001]. The foliar application of 0.5% zinc sulfate in orange trees from November to February increased the quality of flowers [Srivastava 2009].

The study of the effect of zinc on citrus flowering using zinc sulfate at a concentration of 6 g·L⁻¹ at various times indicated that flower shedding in the treatments sprayed in January was greatly reduced and higher yields were obtained in the treated plants [Lolaei et al. 2014]. The effects of urea (0, 600, 900, and 1200 g) as granules in soil, zinc sulfate (0, 5, 7, and 9 g·L⁻¹) as the foliar application on the canopy of

6-year-old sweet lime trees during two years showed that the amount of fruit formation and yield increased with the application of nitrogen and zinc. The soil application of 1,200 g of urea along with seven g·L⁻¹ of the foliar application of zinc sulfate per tree showed the most considerable percentage of fruit formation and the highest yield [Behrooznam and Hassanpour 2005].

Some researchers have pointed to the effective role of zinc sulfate and urea and zinc in increasing the yield of the pomegranate fruit [Sedaghatkish et al. 2014]. The report of Razzaq et al. [2013] on the effects of the foliar application of zinc sulfate on the yield and quality of Kinnow mandarin fruit indicated that the diameter, weight, and amount of ascorbic acid in the sprayed fruit trees increased by 0.6% zinc sulfate, which is consistent with the present study. The results of this study is in line with the report of Khayyambashi et al. [2007] on an increase in soluble solids caused by the foliar application of zinc and iron on pomegranate fruit, that of Behrooznam and Hassanpour [2005] on the effect of the foliar application of zinc sulfate on increasing fruit formation and the nitrogen and zinc consumption on the yield in sweet lime, as well as the results of the study done by Albrigo and Syvertsen [2001] who reported that the use of nitrogen in citrus fruits increased fruit weight, fruit juice, vitamin C and yield, and the results of the study done by Lolaei et al. [2014] on the effect of zinc on the flowering of citrus trees.

CONCLUSIONS

According to the information obtained from the time of the flower induction in the desired area, by considering the time of absorption of effective elements involved in the flower induction in the plant leaves, foliar application can be done so that at the beginning of the flower induction stage, there is no restriction in absorbing the desired elements.

Nitrogen and zinc, because of their various physiological functions in growth and developmental processes, directly and indirectly, affect the vegetative and reproductive growth of citrus trees. In the present study, after the foliar application of urea and zinc sulfate, to a large extent in the combined treatment, all the studied traits were significantly affected compared to the control. Under the conditions of this experiment,

application of urea (3 g·L⁻¹) and the combined treatment of urea (3 g·L⁻¹) and sulfate zinc (5 g·L⁻¹) can be sprayed during the first half of October to improve the quality traits of and the combined treatment of urea and zinc sulfate (both 5 g·L⁻¹) to increase the yield of sweet lime trees.

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