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Review Article

THE STATUS OF CALCIUM IN THE NORTHERN CITRUS ORCHARDS OF IRAN (MAZANDARAN PROVINCE)

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Citrus is one of the most important fruits and is one of the largest fruit industries,widely distributed in the world (Gogoi and Barbora, 2022),cultivated in tropical, subtropical, and temperate regions from latitude 40^0N to 40^0S, and is the world's largest fruit-producing fruit tree with high economic value (Asadi Kangarshahi *et al.,* 2017). According to the latest reports of FAO, the investigation of the area under cultivation and the amount of citrus production shows that the total area under cultivation of citrus orchards in the world is 9898463 hectares and the total global production is approximately 158 million tons (Anonymous, 2021). China, Brazil, India, Mexico, America, Spain, Iran, Egypt, Turkey, and Nigeria respectively are the major exporters of citrus in the world (Anonymous, 2021). With a production of about 5 million tons, Iran ranks seventh in the world, accounting for 3.7% of the world's citrus production (Anonymous, 2021). The total citrus cultivation area of Iran is about 290,000 hectares, 82.8% of which are fertile trees and 17.2% are citrus seedlings. In terms of yield hectare^{-1}, Iran is ranked ninth in the world (Anonymous, 2021). Citrus cultivation regions of Iran can be divided into 3 regions of Caspian Sea coasts, the central region, and Bandar Abbas region, and the Oman Sea. Mazandaran province with 34.6% and 45.1% fertile lands and citrus production had the highest level of citrus cultivation and production, respectively. The citrus cultivation area in Mazandaran province is more than 120,000 hectares and annual production is about 3 million tons, about 60 to 70 % of which is orange, including Thomson Newell (Asadi Kangarshahi *et al.,* 2017). From the viewpoint of soil fertility and plant nutrition, citrus production in Mazandaran province is faced with some problems such as unbalanced use of chemical fertilizers, inappropriate management of fertilizers containing nitrogen, boron toxicity, death of top branches and citrus deterioration and calcium deficiency can be noted (Asadi Kangarshahi and Akhlaghi Amiri, 2018). Calcium deficiency in citrus orchards in northern Iran is a common limitation. Calcium deficiency is usually more common in acidic soils that naturally leach out calcium (Asadi Kangarshahi *et al.,* 2017). The aim of this work was to investigate the status of calcium in the soils of Mazandaran province and the role of calcium in citrus production and disorders related to its deficiency.

Calcium in the soil

Calcium is the fifth most abundant element in the earth's crust (average concentration of 3.6%). Calcium exists in the form of mineral, exchangeable, soluble as well as calcium ions in the soil. The ion form of calcium is available on the exchangeable surface of soil particles or in soil solution. Soluble and exchangeable calcium are the main forms of calcium absorbable in the soil. In calcareous soils, calcium carbonate is the dominant calcium mineral in the soil (Bashour and Sayegh, 2007; Yang *et al.*, 2010). Naturally, calcium deficiency in citrus trees is more in acidic soils with heavy rainfall and irrigation water where natural calcium is leached out, light sandy soils, soils with surface waterlogging, soils with excessive uptake of some chemical fertilizers, soils with high magnesium and potassium, as well as under heat stress conditions, salinity stress can be viewed (Bramlage, 1994; Hirschi, 2004). Furthermore, inappropriate management strategies intensify calcium removal from the root zone soil. Calcium deficiency also develops in soils with excessive magnesium and potassium (Bramlage, 1994;Hirschi, 2004).

Calcium status in the citrus orchards of Mazandaran province

The quantity of calcium carbonate in the soils of Mazandaran province steadily increases from west to east (Fig.1).Also, soil studies of citrus orchards in the east of Mazandaran have shown that the amount of calcium carbonate in the orchards gradually increases from the middle to the east so that the amount of calcium carbonate in Amol and Babol is less than 1% and in the east of Sari and Neka reaches more than 30%. Also, different studies in the eastern regions of Mazandaran confirm the abundance of lime in the eastern region of Mazandaran (Tehrani *et al.,* 2011; Asadi Kangarshahi and Akhlaghi Amiri, 2014).

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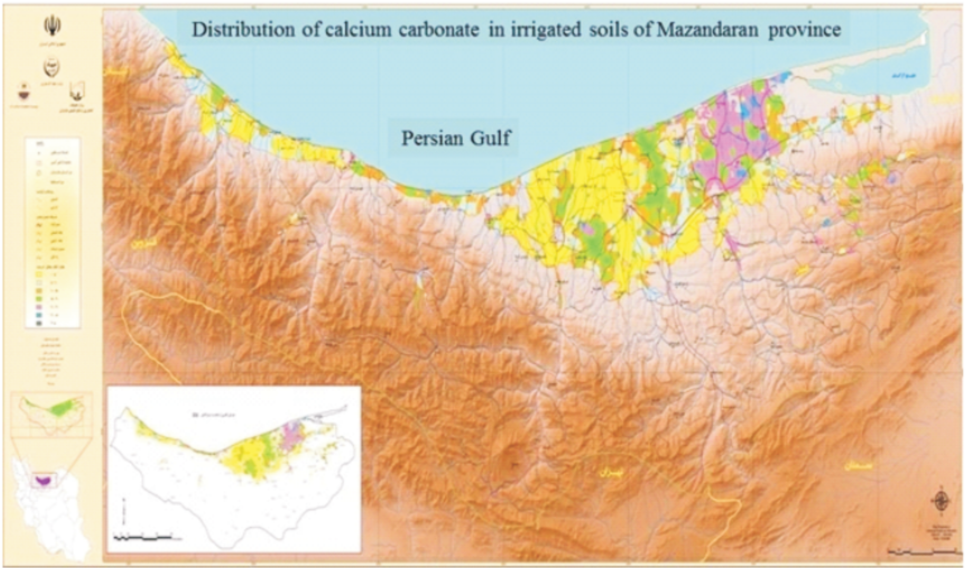

Figure 1. Distribution of calcium carbonate in soils of Mazandaran province. Regions with yellow, 0-5%; light blue, 5-10%; brown, 10-15%; green, 15-20%; pink, 20-30%; dark blue, 30-50% and gray, show more than 50% calcium carbonate equivalent (Tehrani *et al.,* 2011).

Calcium in plants

Calcium is essential for the stability of plasma membranes and cell walls. Within the cell, most of the calcium is in the endoplasmic reticulum and chloroplast (Hirschi, 2004). The dominant part of cytoplasmic calcium acts as a message transmitter and is effective in coordinating cellular responses to development processes and environmental challenges. Calcium is also effective in balancing organic and inorganic anions in vacuoles (Asadi Kangarshahi and Akhlaghi Amiri, 2014; Asadi Kangarshahi and Akhlaghi Amiri, 2018). Developing tissues have an instant and stable need for calcium, which is supplied by the xylem vessels and is highly dependent on transpiration (White and Broadley, 2003). Calcium deficiency disorders occur when enough calcium was not available for the development of developing tissues. Hence, calcium deficiency seems mainly and specifically in the meristem regions where cell division is ongoing and new cells are being produced (Hirschi, 2004; Hopkins and Huner, 2004).

Calcium in citrus

Calcium is the first nutrient in terms of abundance in leaves and the second in terms of abundance in citrus fruit peel, and a large part of this calcium is located in the cell wall (Nagy *et al.,* 1985). Calcium is important in the resistance of citrus trees to diseases due to two key roles in the growth and development of citrus trees (Hirschi, 2004; Marschner, 1995). In general, calcium deficiency reduces or stops the growth of trees, decreases leaf size, diminishes chlorophyll in leaf margins and between main veins, intensifies colorless and leaf abscission, increases root rot, decreases yield, and relatively decreases fruit size (Srivastava and Singh, 2003; Asadi Kangarshahi and Akhlaghi Amiri, 2014). Chlorophyll dimming occurs mostly in winter during leaf margins and between main veins due to calcium deficiency in citrus trees in the northern regions of Iran and generally reduces the power of these trees, reducing yield, leaves tinning, and small thick leaves (Asadi kangarshahi and Akhlaghi Amiri, 2014). Uptake and transfer of calcium in the xylem vessels and its transfer to citrus fruit is associated with the intensity of aerial transpiration and increases with increasing the amount of calcium transpiration to the aerial organs. But the intensity of calcium entry into the fruit depends on the growth stage of the fruit. In the early stages of fruit growth (fruit formation to physiological dropping) due to the high equilibrium of stomata and lack of complete formation of a wax layer on the fruit surface, the intensity of transpiration flow from fruit is relatively high, which increases the transfer and entry of calcium to the fruit, but in general, calcium transfer to fruit may be influenced by the rootstock, fruit or microclimate of the tree as well as the temperature, relative humidity and rainfall of the region (Peryea, 1991; Storey *et al.,* 2002).In different regions of Mazandaran province, due to the lack of sunshine hours, especially in the early growing season, as well as high relative humidity,there is a possibility of calcium deficiency in developing tissues in most years during the development of spring shoots and flowers (Asadi Kangarshahi and Akhlaghi Amiri, 2014). Calcium absorption in the roots of citrus trees is limited to areas of the root where the casparian strip does not exist between endodermic cells or is degraded or endodermic cells do not surround the vascular cylinder,that these areas mainly contain the tip of the roots (White and Broadley, 2003). Calcium is very important in fresh fruits and its amount in fruit is much less than in leaves, the maximum absorption of calcium in citrus fruit is about 45 to 50 days after flowering and after the June drop, the absorption decreases rapidly (Storey and Treeby, 2002).In citrus fruit, most calcium is entered into the albedo tissue in the first step of fruit growth up to about 100 days after flowering, and then calcium is evenly distributed between albedo and fruit meat (pulp) (Storey and Treeby, 2002).There is a time difference between the onset of root growth and the growth of the aerial parts and root growth starts about 15 to 20 days after aerial parts, so in areas, with high relative humidity and rainfall in the early growing season there is a possibility of calcium deficiency and disorders in developing tissues, especially during flowering and fruit formation (Peryea, 1991;Asadi Kangarshahi and Akhlaghi Amiri, 2014).

Changes in calcium concentration in citrus leaves

Calcium concentration in leaves increases with the age of the leaves of trees. So that the concentration of calcium in leaves in mid-June is about 0.88% lower than the leaves in August. Also, with increasing age and diameter of shoots, calcium concentration decreases so that calcium concentration in shoots less than 1 cm is about 1.64% based on dry weight and reaches to about 0.85% in larger branches. On the contrary,the results of calcium concentration in roots showed that calcium concentration in fibrous roots was lower than calcium concentration in shoots. Calcium concentration in fibrous roots less than 1 cm is about 0.86% and in larger fibrous roots is about 0.65% (Fig. 2). The data regarding optimal concentration of calcium in leaves of different citrus cultivars are presented in Table 1. In general, climatic, soil and physiological factors may be effective in calcium deficiency (Asadi Kangarshahi and Akhlaghi Amiri, 2018).

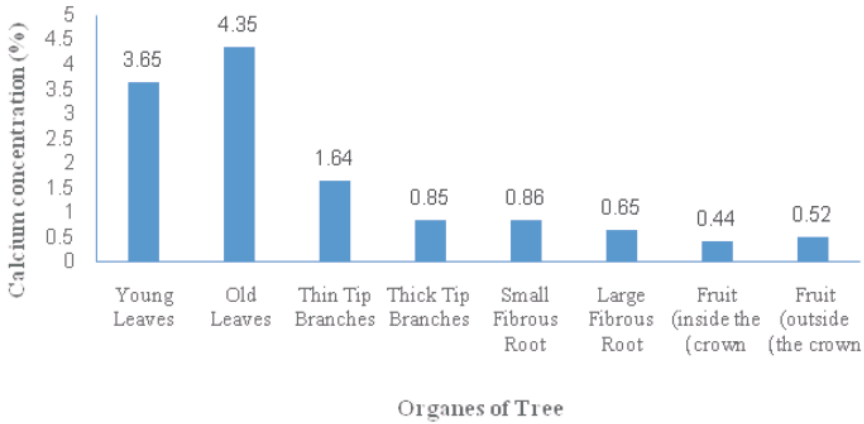

Figure 2. Calcium concentration in different organs of Thomson Newell orange trees

Table 1. Optimum concentration of calcium in the leaf of different citrus cultivars (Asadi kangarshahi and Akhlaghi Amiri, 2014)

Cultivar	Optimum concentration of calcium in leaf (%)
Washington Navel	3.5-5
Satsuma mandarin	3.5-5
Clementine and Page	4.5-4.5
Blood orange and sangin	4.6-8
Valencia	3.5-5
Grapefruit	3.5-5
Lime and Lemon	2.2-3.5

The trend of calcium changes in fruit

The results of measuring the calcium concentration of fruits of Thomson Newell trees in late May in the east of Mazandaran showed that calcium concentration in fruits outside the crown was higher than those in the crown. The results of studying the trend of calcium changes in fruit peel during the growing season showed that calcium concentration in the fruits of the outer part of the crown of trees gradually increased over time and reached the maximum in July and had less variation from July to September, then calcium concentration began to decrease and decreased to the minimum at harvest time (Fig. 3). In the fruits within the crown, the calcium concentration of fruit peel gradually increased after fruit formation. It was maximized in July and then had less variation from July to August and started to decline again from September and was minimized in November (Fig 4). The intensity of calcium entry into the fruit depends on the growth stage of the fruit in the early stages of fruit growth (fruit formation to physiology) due to the high number of stomatates and also the lack of complete formation of wax on the fruit surface, the intensity of transpiration flow from fruit is relatively high which increases the transfer and entry of calcium to the fruit (Hocking *et al.,* 2016; Asadi Kangarshahi and Akhlaghi Amiri, 2018).

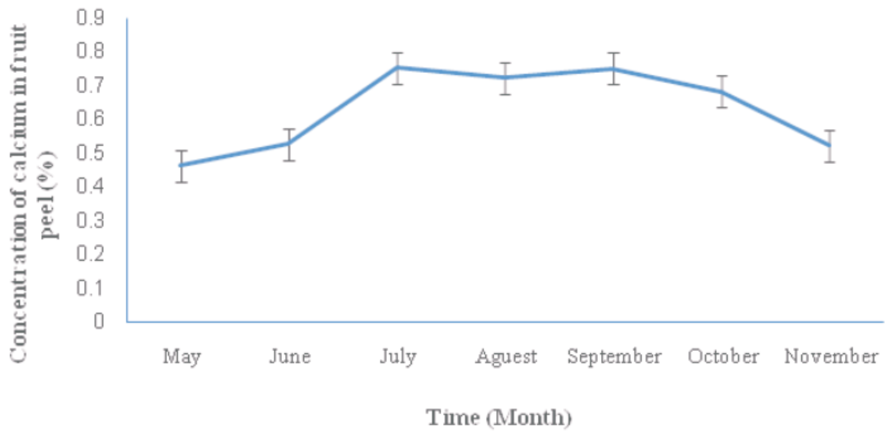

Figure 3. Changes in calcium concentration in the peel of Thomson Navel orange fruit (outside the crown) during the growing season

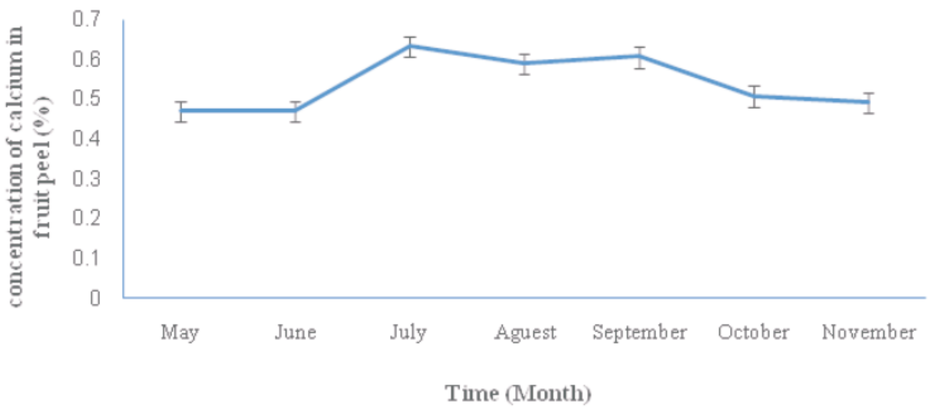

Figure 4. Changes in calcium concentration in the peel of Thomson Navel orange fruit (within the crown) during the growing season

Fruit structure and calcium absorption

Citrus fruit consists of three parts: exocarp (flavedo), mesocarp (albedo), and endocarp (edible part). The structure of the outer layer of an orange peel consists of a leathery exocarp, which is the outermost layer of the outer peel. The leathery exocarp after fruit formation is very thin and then gradually thickens and more continuously and within a short time after summer loss almost this layer is continuous and reaches maximum thickness after that, this surface leathery exocarp limits sweating and thus limits calcium transfer through the xylem vessels to fruit and also absorbs calcium through foliar application. The cross-section of citrus fruit shows that the vascular bundle is mainly located in the inner part of the fruit peel and by limiting or stopping transpiration from the fruit surface, almost the transfer of water and nutrients through the xylem vessel is also minimized and the metabolites and water required for the fruit will be provided more through phloem vessels. In general, due to the inability to transfer calcium in phloem vessels, the transfer of calcium from roots and xylem vessels to citrus fruits is minimized and stopped after the summer fruit drop. Also, due to the continuity of this leathery exocarp and its thickness, the direct entrance of nutrients through direct spraying to the fruit surface will be minimized. Therefore, calcium spray at the beginning of fruit development and the early second stage of fruit development can be effective in increasing the calcium content of the fruit, and over time after the early second stage of fruit growth, gradually the calcium absorption efficiency will be reduced and will not have much effect on increasing fruit calcium (Srivastava, 2012; Asadi Kangarshahi, 2018).

Physiological disorders and stresses related to calcium

Calcium and fruit cracking

The cracking between citrus fruits and other fruits is different. This difference is due to the specific morphology of citrus fruit, which includes an endocarp and exocarp, the exocarp consists of two parts: an inner white layer or albedo, and an outer layer or flavedo. During the first stage of fruit development (cell division stage), the majority of flavedo cells are formed and then flavedo cell division decreases, and in contrast to meat cells in the second stage of fruit growth, they begin to develop. The pressure caused by the rapid development of fruit meat causes fine cracks and the onset of cracks in the cream or navel of the end of the fruit, where the skin is thinner and its structural strength is also less than other parts of the peel. Some environmental factors can cause weaker peel development and make the peel more susceptible to cleavage (Fig. 5 A). These factors include an imbalance of nutrients, especially potassium and phosphorus, hot and humid climatic conditions, irregular irrigation, and high crop yield. However, it seems that stress during the first stage of fruit development(II), which is the majority of flavedo cells and their constructional continuity is determined, is the most important determinant of fruit susceptibility to fruit ripening. High yield (a large number of fruits), inappropriate irrigation, as well as inappropriate and inadequate fertilization during this critical period, significantly increase the ripening potential of fruit (Bergmann, 1992; Asadi Kangarshahi and Akhlaghi Amiri, 2011).

Calcium and fractures of the inner skin or creasing

Calcium attached to the pectate in the middle layer is essential for the strength and firmness of the cell wall (caused by the interlinks of the middle lamella pectic chains) as well as gelatinous pectic properties. Calcium also acts as a cofactor in enzymatic changes and conversions, osmotic regulators, and secondary messages in enzymatic reactions. In calcium deficient tissues, poly galactokinase activity increases and increasing poly galactokinase causes degradation of cell walls, and destruction of affected tissues, fracture of the internal peel tissue of citrus fruit(Figures 5- B and C) (Grierson, 1981; Storey *et al.,* 2002; Asadi Kangarshahi and Akhlaghi Amiri, 2011).

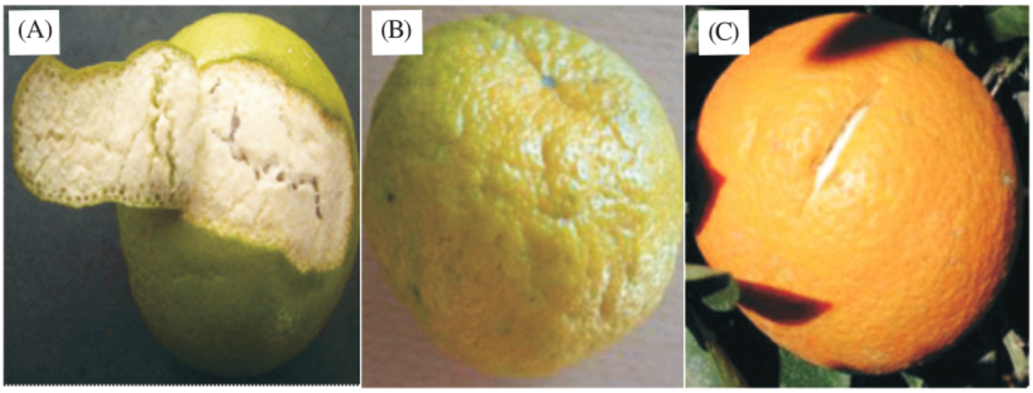

Figure 5. Cracking in Valencia orange (A), Creasing in clementine tangerine (B) and creasing in Washington Navel orange (C)

Calcium deficiency increases the sensitivity of trees to cold and freezing stress. Calcium is an essential element for closing apertures due to cold and freezing stress in tolerant genotypes. Increased calcium intake enables faster closure of apertures, and this effect is very clear in trees exposed to cold and freezing stresses. Also, the closure of apertures due to abscisic acid (ABA) is partly due to calcium released from the reserves of aperture or apoplastic guard cells, and this action has caused calcium as one of the most important elements in the tolerance of trees to frostbite. In general, increasing the amount of calcium during cold and freezing stress is necessary to respond to cold and freezing stress. Most studies have shown that calmodulins are effective in inducing cold stress-tolerant genes. In summary, one of the most important responses of plants to cold and freezing stress is to increase the amount of calcium cytosol. Field studies have also shown that the damage of citrus trees with calcium deficiency is much higher during cold and freezing stress than trees with optimal calcium in leaves (Asadi Kangarshahi and Akhlaghi Amiri, 2014).

Calcium and fungal diseases

In general, calcium increases tissue resistance to infection of pythium, sclerotinia, botrytis, and fusarium fungi. Huanglongbing (HLD), known as citrus greening disease, is present in many citrus regions of the world, including southern parts of Iran. Calcium concentration in the leaves of trees infected with HLB is lower than in healthy trees. In addition, calcium may neutralize the negative effects of HLB on the root growth of infected trees. There is some evidence that optimal nutrition can reduce HLB symptoms and it seems that calcium nutrition management is effective in increasing productivity and increasing the economic life of trees (Rahman and Punja, 2002; Gottwald *et al.,* 2007). Calcium directly affected on resistance of leaves, fruits, roots, and other plant tissues by improving cell mechanical strength, cell membrane strength, and cell wall strength. A large part of calcium (unlike other high-consumption nutrients) is located in the cell walls of plant tissues due to its high position in cell walls as well as very low calcium transfer from the cytoplasm membrane and its entry into the cell cytoplasm. In the middle lamella of the cell wall, calcium sticks strongly to the carboxyl groups of galacturonic acid (pectins) and becomes almost non-exchangeable. In most dicots, including citrus, approximately 50% of calcium is in the form of calcium pectate. The degradation of pectates is carried out by poly galactoronase enzyme and high concentrations of calcium in tissues severely prevent the activity of this enzyme. Therefore, the amount of calcium pectate in the cell walls of plants and fruits is very effective in the sensitivity of these tissues to fungal infection. So that the higher the amount of calcium, the less sensitivity of tissues to fungal infection(Taylor and Locascio, 2004).

Calcium and drought stress

The effect of calcium on increasing tolerance to drought stress due to the signaling roles of calcium in controlling the movement of apertures was reported in previous studies. For stable openness of stomatates, calcium must be removed from the cytoplasm of the guard cells, but the closure of stomatates with high calcium in the cytoplasm is stimulated. The effect of calcium on water efficiency is not only limited to regulating the activity of stomatates, but also calcium is essential for the development of healthy roots. Root growth is greatly reduced under calcium deficiency conditions and stopping root tip growth is caused by severe calcium deficiency conditions, leading to a poor root system. Weak and shallow roots cannot effectively absorb water from the soil, leading to high water losses in the form of drainage and reduced water use efficiency. Calcium stimulates the vegetative power of the roots, which in turn creates a higher specific level for nutrient absorption (Asadi Kangarshahi and. Akhlaghi Amiri, 2014; Eticha *et al.,* 2017).

Therefore, the balanced nutrition with right dose, right time, right method, right source and right ratio is an important key to achieving a stable condition (Patel *et al.,* 2021).The most suitable concentration of calcium nitrate for foliar application was 3-5 kg1000 liter^{-1}. Foliar application of calcium nitrate after fruit set and before physiological drop (June drop) in increasing calcium in fruit peel is much more effective. The most suitable calcium foliar time to increase calcium concentration in orange fruit, from fruit set to onset of physiological drop (the first phase of fruit growth). Therefore, foliar application of calcium nitrate after fruit set and before physiological drop (June drop) is strongly recommended. Due to the acidity of the soil in Mazandaran province, it is important to pay attention to plant nutrition. As a result, calcium can easily leach and become inaccessible to plants. Calcium is an essential nutrient in maintaining cell wall strength and fruit structure and extends the shelf life of orange fruit due to ranges having a specific wax layer, it is necessary to pay attention to the time of foliar fertilization to provide the necessary calcium content for the plant. Considering the numerous other roles of calcium in the management of biotic and abiotic stresses, plant nutrition management in Mazandaran province is very important in order to provide the required amount of calcium to citrus trees.

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