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Effects of super-absorbent nano polymer, complete Gromor fertilizer, iron, and zinc on Hecogenin processing in *Agave Marginata* L. in the climatic conditions of Shiraz, Iran

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ABSTRACT

This study was carried out in flowerpots within the framework of a completely randomized design with eight treatments. Statistical data analysis was performed using SAS ver. 9, and the charts were drawn in Excel 2016. One of the aims of this study was to investigate the effect of super-absorbent nano polymer, complete Gromor fertilizer, iron, and zinc on the quantitative and qualitative performances of *Agave marginata* L. in the climatic conditions of Shiraz City, Iran regarding one of its biologic products called hecogenin. The results of the analysis of variance of the effect of treatment on hecogenin in the first year were significant at the statistical levels of 5% and 1%. The same analysis for the second year produced significant results at the statistical levels of 5% and 1%. In the first and second years, the application of different treatments caused a significant reduction in hecogenin concentrations. Furthermore, dry plant weight had significant positive correlations with dry matter percentage and hecogenin concentration ($p < 0.01$). Hecogenin is derived from the leaves of *A. marginata* after are extracting sisal fibres. This substance is used in pharmacy to produce cortisone and sex hormones.

Keywords: *Agave*, Cortisone, Hecogenin, Micronutrient, Sex hormone.
Article type: Research Article.

INTRODUCTION

The global approach to the use of medicinal plants has attracted the attention of many researchers (Sharifi Hosseini *et al*. 2018; Rakhimova *et al.* 2021; Kamali Omidi *et al.* 2022; Hasan *et al.* 2022; Baniesmaeeili *et al.* 2023; Manouchehri 2023; Shafiq *et al.* 2023; Mandil *et al.* 2023; Mheidi *et al.* 2023). In recent years, the efforts of numerous scientists with various disciplines, including agricultural botany, biology, chemistry, pharmacy, biotechnology, medicine, and other related ones, have been focused on scientific research to establish the related industries and spreading the public culture of using herbal medicines and medicinal plants (Manouchehri *et al*. 2022; Bazari Moghaddam *et al*. 2022; Soltanbeigi *et al*. 2023; Sulieman *et al*. 2023). Nowadays, it is a recognized fact that herbal medicines, due to their natural source and compatibility with the physiology of the human body, are less dangerous and have less side-effects than chemical drugs. These characteristics are among the main reasons for the renewed tendency of the world population toward the use of herbal medicines instead of synthesized and chemical drugs. Herbal medicines are among the rich resources of any country, which can be exported as well. In terms of weather conditions and geographical location, Iran is considered as one of the best regions in the world for the growth of these types of plants, and has been a source for the production of medicinal plants. There is an increasing trend to the active ingredients of medicinal plants in the pharmacy and food industry (Abbasi *et al.* 2014, 2020; Karimi *et al.* 2019; Aidy *et al.* 2020). The active ingredients extracted from these plants are currently being used in the beverage, canning, and confectionary industries in order to improve the taste, colour, and smell of the products (Omidbeigi 2005). As a medicinal plant, Agave is of great significance. The term "Agave" is derived from the Greek word "Agavos", meaning "illustrious" (the plant becomes exceedingly beautiful and spectacular when flowering). In some regions of the world, this plant is wrongly called "Aloe" due to its similar appearance to the plant Aloe (of the Tulip family). Formerly, Agave was considered to be a member of the family Daffodils or Amaryllicaceae (Mortezaeinejad 2011). However, it is currently considered to be a member of an independent family, Agavaceae (Mozaffarian 2012). This family comprises 300 permanent species. This is an extremely slow-growing plant that often does not flower, and dies after flowering. *Agave marginata* L. is a plant with thick green leaves and yellow margins. If cultured in an appropriate environment, it grows and reaches a height of over one meter, however, cannot grow in the shade. It requires soil moisture and is drought-resistant. This plant can be fed 3 g L^{-1} iron and zing once every two weeks from April to September. Propagation can be done both using seeds and planting the basal shoots near the roots. Although, due to segregation, propagation through seeds is not recommended as a suitable method. This method can be used for breeding studies (Brickell & Cathey 2004). The applications of Agave in the field of medicinal plants and other fields are noteworthy: Hecogenin is derived from the leaves of Agave after extracting its sisal fibres. This substance is used in pharmacy to produce cortisone and sex hormones (Ahmadzadeh & Rostami 2016).

MATERIALS AND METHODS

The use of micronutrients in plant feeding is one of the methods applied in developed countries to achieve sustainable development and food security. A management tool that can improve plant growth and increase production is feeding the plants and fertilizing the soil through the optimal use of the required nutrients. In proper plant feeding, not only should each element be sufficiently made available to the plant but also their cooperation or antagonism within the plant structure should be taken into account (Malakouti & Gheibi, 2000; Marschner, 1995). Regarding the significance of the elements used in plant feeding in the current research, it should be noted that most arable soils in Iran have zinc deficiency. The deficiency of zinc in soils is evident for multiple reasons, such as calcareous soils, high acidity, and the presence of sodium bicarbonate in irrigation waters. Zinc is a building block in the structure of a large number of enzymes, and plays an important role in the biochemical transportations of cells. Zinc deficiency eventually causes a reduction in the activity of growth hormones. Iron is an important component of plant enzymes, such as electron-acceptor enzymes, and plant chlorophyll production. To use this element, iron chelates should be utilized, the most famous of which is EDDHA chelate, which can meet a plant's iron needs under irrigation conditions. This element along with zinc and manganese play a significant role in the production of secondary metabolites in various plants (Marschner 1995; Malakouti & Gheibi 2000). Given the importance of the subject, a research gap is evident in this area. Plant nutrition is a critical issue that has a special place in the current research. The use of high- and low-consumption elements in plant feeding at different levels indicates a novel initiative in this research. There is not any difference between planting medicinal and aromatic plants in order to extract their active ingredients and planting herbaceous plants. The active ingredients make up a relatively small portion of the dry plant weight, between 0.1 – 2%. The properties of these active ingredients are affected by processing processes. The use of high-consumption elements and micronutrients in this study will generally have a significant effect on the processes of secondary metabolites and active ingredients. According to the literature, about 0.065% hecogenin is found in the leaves of the *Agave americana* species grown in India. Another report on *A. cantala* decomposition states that the leaves of the plant contain the steroids sapogenin and hecogenin. The latter is obtained from the remains of the plant leaves after are extracting its sisal fibres. Sisal is a Mexican word referring to strong fibres derived from the leaves of agave and used in the textile as well as rope industries. In addition, the plant itself is also called sisal. Furthermore, the leaves of the plant contain saponases as well. Another report states that during the decomposition of *A. sisalana*, hecogenin is obtained from the remaining pulp of the leaves after the leaf fibres are separated. Hecogenin is used in pharmacy to produce cortisone and sex hormones (Mirheydar 2010).

In the current research, the following treatments were used for plant feeding:

1. Iron chelate fertilizer containing 6% water-soluble iron with the formulation Sodium Ferric Ethylenediamine bis (FeEDDHA), 2-Hydroxyphenyl acetate in the form of microgranules produced in the UK;
2. Iron fertilizer in the form of a reddish powder, completely water-soluble;
3. Water-soluble Gromor 20-20-20 (N.P.K) fertilizer;
4. Water-soluble Gromor 15-15-30 (N.P.K) fertilizer;
5. Water-soluble Gromor 12-12-36 fertilizer;
6. Water-soluble super-absorbent nano-polymer;
7. Zn fertilizer containing 6% water-soluble zinc.

Table 1. Analysis of Gromor fertilizers.

Row	Fertilizer	Total nitrogen (%)	Nitrate nitrogen (%)	Ammoniacal nitrogen (%)	Nitrogen urea (%)	Absorbable phosphorus (P_2O_5; %)	Potassium (K_2O; %)
1	20-20-20	20	5.9	3.9	10.2	20	20
2	30-5-15	15	9.1	2	3.9	5	30
3	36-12-12	12	3.8	4	4.2	12	36

Proper and principled plant feeding procedure in research is based on four main principles:

1. Right kind of fertilizer;
2. Right amount of fertilizer;
3. Right time;
4. Right application.

In the science of plant nutrition, this is called the R4 law. Failure in each of these principles will prevent us from achieving the desired results. The first principle is in the hands of the producers, while the other three are in the hands of recommenders. The fourth principle in particular is in the hands of farmers, and neglecting to execute it would compromise the first principle as well.

The fourth principle of the plant feeding law, which is fertilization, is carried out through one of the following 5 methods:

1. Application by broadcasting across the soil surface;
2. Application through the soil in bands;
3. Application through the soil in pellets;
4. Application through irrigation systems;
5. Application by spraying fertilizer solutions on the foliage of plants.

Fertilization through irrigation systems is also called Fertigation. Regardless of the method used for plant feeding, the nutrients should be solved in water and ionized in order to turn into absorbable ions. Through this method, the task is inevitably carried out, and the combination of water and fertilizer would ensure the absorption of nutrients to a higher degree (this method is utilized in the current study).

In general, some of the advantages to fertigation are as follows:

1. Fertilizer loss is greatly reduced
2. Fertilization will be continuous and timely
3. Dissolved fertilizer will reach the plant
4. There will be no phytotoxicity (Khoshgoftarmanesh, 2010)

Hecogenin measurement

To measure the hecogenin levels, one gram of agave leaf powder (dried in a vacuum oven at 40 °C) was thoroughly mixed with 3 mL methanol (Merck, Germany) and homogenized for 3 min. It was then centrifuged (at 5000 rpm for 5 min) and filtered using a 0.22-µm filter. Hecogenin was measured using a plate reader (Citation 5, USA) at 255 nm wavelength. The wavelength of 255 nm was selected based on the standard sample scan within the range of 190 – 700 nm. A standard hecogenin diagram was drawn using pure hecogenin (Merck, Germany) at concentrations of 0 – 5 μg mL^{-1}.

RESULTS AND DISCUSSION

Using high-consumption elements and nutrients in this study generally exhibited a significant impact on the processes of secondary metabolites and active ingredients. In literature review, regarding to the protection of agave leaves in bioethanol, a study was carried out in Mexico exhibiting that 2% sulfuric acid treatment produced the highest amount of sugar content from this plant in 60 min and at 120 °C (Rijal *et al.* 2016). In addition, based on the extraction of fibres from agave leaves through various solvent treatments, including acetone, hydrochloric acid, sulfuric acid, etc., a number of studies were conducted in Spain and Mexico, in which the most significant effect was observed by the hydrochloric acid mixture (Hernández-Hernández *et al.* 2016). In the case of agave bagasse extract with pretreatment of calcium oxalate, using the unique solution and alkaline hydrogen peroxide in studies carried out in Mexico and the United States, it was found that the highest amounts of xylene and glucan were obtained by pretreatment of this unique solution using the bagasse extract of this plant. Moreover, given the effects of various types of tannins, alkaloids, sugars, and sodium caseinate on the bulk and size of different parts of the plant and its morphological traits, it was found that the most significant effects were attributed to those of agave fructans and sodium caseinate (Sosa-Herrera *et al.* 2016). A phytochemical study of the biostimulants of agave saponins has shown that extraction can be carried out through various techniques (ultrasound-assisted, Soxhlet, maceration, microwave-assisted). However, the amount of active ingredients obtained from each of these methods has been different (Sidana *et al.* 2016). Based on the determination of the genomic structure of DNA ribosomes in agave by PCR, it has been found that this structure has useful nucleotide sequences for production and transfer to the genetic structure of other plants of the same family, which can be used in breeding programs (Rubio-Piña *et al.* 2016). Generally, micronutrients play an important role in the production and function of medicinal plants, including iron, which is involved in the structure of cytochromes, chlorophyll production, and oxidation – reduction operations. Soils with a pH greater than 6 are usually iron-deficient (Marschner 1995). Zinc is an important element in the activity of vital enzymes, growth regulators, photosynthesis, early plant growth, nitrogen fixation, grain protein, and yield. Zinc deficiency, due to its adverse effect on biosynthesis of auxin, can reduce stem height and plant yield (Ram *et al.* 2002). Numerous works have been carried out on the nutrition of other medicinal plants, such as *Melissa officinalis* L. The effects of the foliar application of micronutrients (Cu, Mn, Fe and Zn) on the essential oil and the morphological and physiological characteristics of this plant were investigated and it was determined that the dry and wet weights of branches and roots, number of flowers, dry weight of dried flowers, amounts of flavonoids and phenols, and quantity and quality of the essential oil were significantly affected by the treatments used, and a significant superiority was observed over the control treatment (Yadegari 2017a). By working about the effects of manganese and copper on the volatile compounds of lemongrass, it was found that the highest amount of the active ingredients of neral and geranial extracted in copper and manganese treatments was 150 ppm in two cultivation seasons (Yadegari 2016). In another study, it was reported that the foliar application of 400 mg L^{-1} iron, zinc, copper, and manganese from the time of growth to harvest of the medicinal plants such as borage, marigold, and thyme produced the highest quantitative and qualitative amounts of essential oils and extracts during two cultivation seasons (Yadegari 2015). In addition, it was found that in terms of micronutrients and irrigation cycle for the medicinal plants such as alyssum, thyme, and calendula, the foliar application of iron and zinc at a concentration of 400 mg L^{-1} with an irrigation cycle of once every three days had the highest effectiveness (Yadegari 2017b). Other factors that can lead to a higher production of essential oils and active ingredients include a cool climate and the presence of sufficient amounts of high-consumption elements and micronutrients in the soil during the growth of various vegetative structures in medicinal plants such as spear thistle (Amiri *et al.* 2018), artichoke (Yadegari & Yousefi 2016), stachys (Alimohammadi *et al.* 2017), and sage (Reaisi *et al.* 2019). Furthermore, the application of the micronutrients such as zinc and iron has led to an increase in camazoline in chamomile (Nasiri *et al.* 2010) as well as an elevation in the active ingredients and qualitative essential oil yield in basil (Said-Al Ahl & Mahmoud 2010). Nutrition management is one of the most important factors determining plant quantity and quality. The deficiencies, such as unable to recognize the true nutritional needs of the plant nutrients in similar nutritional management conditions or the relationship between the nutritional factors and eco-physiological conditions of its breeding areas and the characteristics and growth deficiencies of various agave, has led to plant loss. This has prevented the cultivation of suitable indigenous, medicinal, and commercial cultivars in fertile areas due to the lack of familiarity to their nutritional needs in specific eco-physiological conditions (Tadayon 2009).

Table 2. Treatments for hecogenin in the first year.

TR	Mean
Control	28.95
British iron chelates	21.61
Iranian iron fertilizer	19.37
Level 1 Gromor	21.82
Level 2 Gromor	27.61
Level 3 Gromor	29.62
Zinc fertilizer	30.20
Super-absorbent nano-polymer	29.01

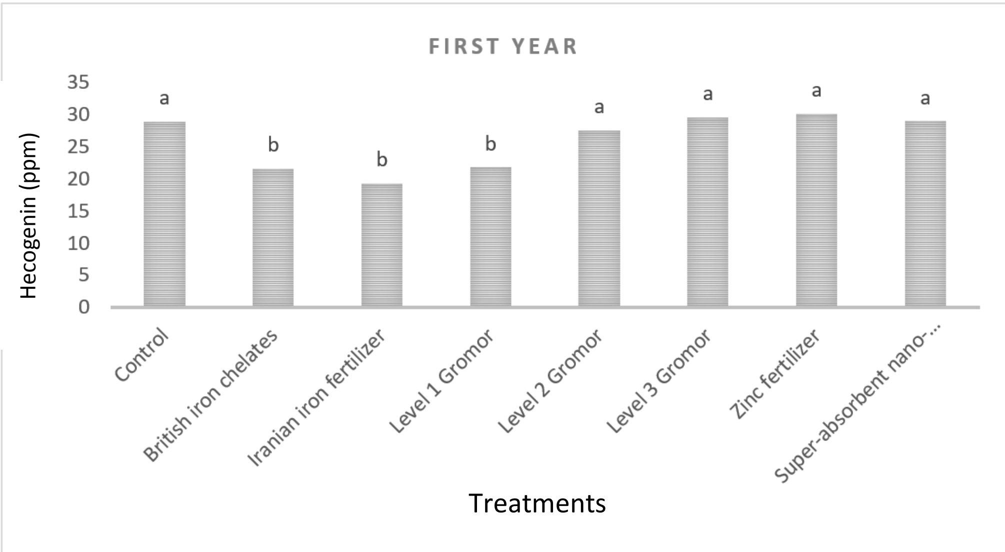

Fig. 1. Hecogenin diagram in the first year. Dissimilar letters show statistically significant differences about $p < 0.05$.

Table 3. Treatments for hecogenin in the second year.

TR	Mean
Control	29.24
British iron chelates	14.32
Iranian iron fertilizer	13.81
Level 1 Gromor	16.08
Level 2 Gromor	21.20
Level 3 Gromor	19.54
Zinc fertilizer	22.34
Super-absorbent nano-polymer	19.57

The results of analysis of variance showed that the effect of the treatment on hecogenin was significant at the 1% level in the first and second years (Tables 2 and 3). Based on a comparison of means, in the first year, British and Iranian iron chelate and level 1 Gromor treatments exhibited significantly lower hecogenin concentrations than the control (28.95 ppm). Moreover, the results did not display any significant difference between the control and other treatments in terms of hecogenin concentration (Fig. 1). The Fig. reveals the mean comparison of treatments on hecogenin in the first year. Mean values named with different letters have significant differences with each other at the 5% level according to Duncan's test. In the second year, the application of different treatments caused a significant reduction in hecogenin concentration in comparison with the control (29.24 ppm; Fig. 2). Hecogenin concentration was lower in Iranian iron fertilizer treatment (13.81 ppm) compared to other ones. Although, the difference in British iron chelate treatment (14.32 ppm) was not significant at the 5% level of Duncan's test (Figure 2).

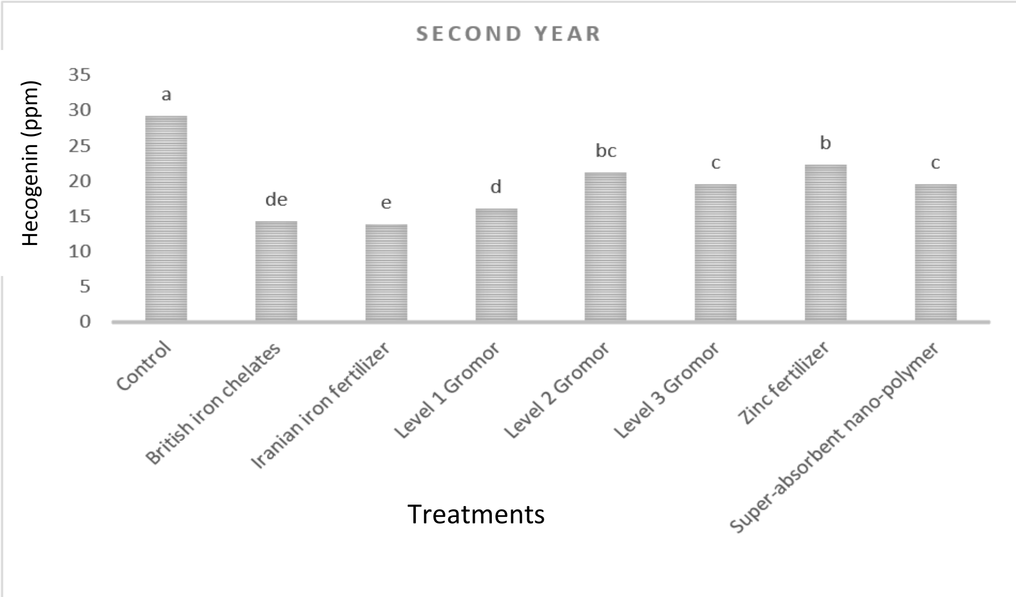

Fig. 2. Hecogenin diagram in the second year. Dissimilar letters show statitically significant differences about $p < 0.05$

CONCLUSION

In terms of genetics, in the agave treasury, which were planted in large flowerpots, simple and uniformly green agaves emerged among the particolored agaves. Among the pots under study (vase, ceramic, white plastic, and black plastic), plant growth was best and most evident in vases with a width of 25 cm and a height of 30 cm. Nutrition management is one of the most important factors determining plant quantity and quality. The nutritional deficiencies, such as not recognizing the true nutritional needs of the plant nutrients in similar nutritional management conditions or the relationship between the nutritional factors and eco-physiological conditions of its breeding areas as well as the characteristics and growth deficiencies of various agave, has led to plant loss. This has prevented the cultivation of suitable indigenous, medicinal, and commercial cultivars in fertile areas due to the lack of familiarity with their nutritional needs in specific eco-physiological conditions (Tadayon 2009). The topic of nutritional safety, which is one of the components of sustainability development, fortunately has been highly regarded by policy makers, planners, and nutritionists in Iran in the past decade, and some progresses have also been made in this area. Reaching the scientific concept of nutritional safety, which is to meet the subcellular nutritional needs of cells, can only be achieved through the application of novel technology in the agricultural sector. The use of micro-elements (micronutrients) in plant nutrition is one of the methods used in developed countries to achieve sustainable development and nutritional safety (Abbasi 2005). Fertilization through irrigation systems is also called Fertigation. Regardless of the method used for plant feeding, the nutrients should be solved in water and ionized in order to turn into absorbable ions. Through this method, the task is inevitably carried out, and the combination of water and fertilizer would ensure the absorption of nutrients to a higher degree (this method is utilized in the current study). In general, applied fertilizer loss is greatly reduced. Applied fertilization will be continuous and well-timed. Dissolved fertilizer will reach the plant. There will be no phytotoxicity (Khoshgoftarmanesh, 2010). Agave plants, due to their medicinal properties, have been used since ancient times. The hecogenin extracted from agave is a steroidal sapogenin with a variety of pharmacological activities, and is an important precursor to steroid hormones. For agave experts, chemical taxonomy has advantages over molecular taxonomy. The presence of specific secondary metabolites and the unique structural aspect of natural plant products provide an important tool for chemical taxonomy. The bioactivity of agave products has turned them into safe and attractive treatment options. In Arizona, USA, large areas are dedicated to the cultivation of agave species. Various parts of this plant are utilized.

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