

Beneficial effects of foliar application of organic chelate fertilizers on French bean production under field conditions in a calcareous soil

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Key words: Aminochelate, amino acid, calcareous soil, nutrient uptake, pod yield, quality.

Abstract: Aminochelate are organic-based chelate fertilizers with higher efficiency for agricultural applications. In the present study, foliar application of three organic-based chelate fertilizers, a macro-micro mixture and soil applied NPK were evaluated on French bean growth characteristics under open field in a calcareous soil. The results showed that plant growth, pod yield (79%) and pod quality were improved by application of chelate fertilizers. Growth parameters as plant height, number of leaves and lateral shoots, shoot dry weight, pod number and pod length were significantly increased by foliar application of the chelate fertilizers. The concentrations of nitrogen, potassium and iron in pods and above all in leaves were increased by foliar application of chelate fertilizers compared to control and soil applied NPK. Pod pH and TSS were not influenced by treatments; however, foliar application of the chelate fertilizers resulted in higher titratable acidity (40%), vitamin C (112%) and protein (35%) content of pods. The results indicate that organic-based chelate fertilizers can be effective safer alternatives for simple chemical salts in calcareous soils.

1. Introduction

Calcareous or lime soils are the dominant type of soil in many parts of the world, and are characterized by high pH as well as high levels of carbonates and bicarbonates. Nutrients and particularly iron uptake in such soil conditions is restricted. In many cases lime-induced chlorosis refers to iron deficiency (Souri, 2015). The leaf chlorosis is mainly due to high pH of soil solution and sap solution induced by high concentrations of carbonates and bicarbonates, which results in precipitation of nutrients in soil and cell apoplast (Mengel, 1994; Nikolic and Römheld, 2002). Plant cultivation in calcareous soils requires especial management techniques and strategies particularly regarding micronutrients supplementation (Souri, 2015). Various fertilizers, as well as different forms of nutrient elements do not have the same uptake efficiency (Jeppsen, 1991; Fernández and



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All relevant data are within the paper and its Supporting Information files.

Competing Interests:

The authors declare no competing interests.

Received for publication 3 November 2017 Accepted for publication 20 March 2018 Ebert, 2005). Since decades chelate fertilizers have been used due to their better suitability and efficiency to meet plant's need of nutrient elements particularly under calcareous soil conditions. Simple nutrient salts are in inorganic form, which for utilization by plant roots must be restructured to ionic form in soil solution. This makes them very vulnerable to various inactivation processes, resulting in their low efficiency rate (Mengel, 1994; Nikolic and Römheld, 2002; Souri, 2016). On the other hand, high price of commercial synthetic chelate fertilizers such as EDTA or EDDHA restricts their application by many farmers. In addition, there is also great doubt on their impacts on plant, environment and ecosystem health issues (Souri, 2015). Aminochelate fertilizers are claimed as suitable alternatives for simple salt fertilizers. For nutritional purpose the safety of fertilizer products is very important. Organic chelates such as aminochelate fertilizers are formulated mainly for foliar application, even if in various studies their soil application also resulted in higher growth compared to soil applied NPK (Souri and Yarahmadi, 2016) or a more complete fertilizer (Salwa, 2011; Garcia et al., 2011). The method of fertilizer application also plays an important role in uptake efficiency by plant roots (Fernández and Ebert, 2005). There are generally two methods of soil and foliar application for most fertilizers. Most of micronutrients and nitrogen fertilizers including amino acids can simply be applied to leaves with acceptable uptake efficiency (Jeppsen, 1991; Marschner, 2011). Nevertheless, foliar application cannot fully replace soil application of fertilizers in agriculture (Souri, 2015; Dehnavard et al., 2017).

Aminochelates are composed of amino acids and a single or several nutrient elements (metals) and represent a more suitable form of fertilizers for sustainable production than routine fertilizers (Souri, 2015). In cultivation systems, application of nitrogen and micronutrients needs a precise and intelligent management, in which various organic chelates can play important role. However, general chemical properties of various aminochelate fertilizers have not been yet studied in soil or within the plant tissues. In recent studies, it was shown that application of amino acid chelates of nutrients in nutrient solution or on plant foliage, significantly increases plant growth and biomass production (Zeid, 2009; Ghasemi et al., 2014; Souri et al., 2017), leaf number, leaf area, leaf SPAD index (Garcia et al., 2011; Souri and Yarahmadi, 2016), fruit yield (Naseri et al., 2013; Pourebrahimi, et al., 2013; Fahimi et al., 2016), fruit quality (Machado et al., 2008; Souri et al., 2017) and

composition of plants (Zhou *et al.*, 2007; Garcia *et al.*, 2011; Ghasemi *et al.*, 2014).

Moreover, application of organic-based fertilizers can also result in higher soil microbial activity and fertility, whereas chemical forms of fertilizers with higher salt effects generally reduce soil microbial activity and soil fertility (Salwa, 2011; Souri, 2015). It was shown that soil application of amino acids has suppressive effect on some soil pathogens such as *Meloidogyne incognita* (Saeed *et al.*, 2005). Green bean is a relatively sensitive plant to lime soil, showing dwarf growth and low yield due to restriction of flower development and fruit set. In the present study effects of foliar application of three organicbased chelate fertilizers were investigated on French bean growth characteristics and pod quality factors under calcareous soil conditions.

2. Materials and Methods

Experimental site

This study was performed at Faculty of Agriculture, Tarbiat Modares Uni., Tehran-Iran during 2013. The field in which the experiment was conducted had a calcareous soil. The soil sample was collected from three parts of the field (0-30 cm depth), mixed together and analyzed for some physicochemical characteristics that are presented in Table 1.

Application of treatments

The experiment was arranged in complete randomized blocks using six treatments and four replications. Treatments were: 1) Control (without any fertilizer application), 2) Soil application of NPK, 3) foliar application of Biomin (amino acid based fertilizer; Arbico-Co, Texas, USA), 4) foliar application of Humifolin (humic acid based fertilizer; Tradecrop Co., Spain), 5) foliar application of DelfanPlus (only amino acid; Tradecrop Co., Spain) and 6) foliar application of a mixture of macro-micro solution. Each replicate

Table 1 - Some properties of the soil used in the experiment

Physico-chemical caracteristics	Data
Texture	Loam-clay
рН	7.8
EC dS/m	2.6
Total C (%)	0.77
Total N (%)	0.09
CaCO ₃ (%)	5.5
Extractable P (mg kg ⁻¹)	15.2
Exchangeable K (mg kg ⁻¹)	250

was a plot of 0.7×1m consisting of 12 plants. Green bean seeds (*Phaseolus vulgaris* L) were directly sown in the soil, and after germination they were thinned to 12 plants per plot. Throughout the growing period all plots and plants were treated the same regarding irrigation, and pest-disease control.

In NPK treatment, a final amount of 6 g per plant from a 20:10:20 formulation was used in three split applications that were incorporated into the soil just near the root systems using 200 ml water. The first application was before sowing and the rest was applied in one week interval after second week of emergence. Foliar application of organic-based and mix fertilizers were done five times during growth period, in a constant concentration of 0.2%. Plants were foliar sprayed at 6-7 o'clock in the morning using a portable sprayer, by which the upper and the lower surface of leaves were treated. The first spray was done at four leaf stage, and the remaining foliar sprays were applied at one-week intervals. All the organic-based commercial fertilizers used in present study were in liquid form, consisting of one or several nutrient elements.

Composition of fertilizers

The composition of various fertilizers is presented as follows: <u>BIOMIN</u>: a liquid fertilizer containing 2% N, 2.5% Zn, 1.5% Mn, 1% Fe, 0.4% Mg, 0.4% Cu, and 0.02% Mo; HUMIFOLIN, a liquid fertilizer containing 42% organic compounds including 37% fulvic and humic acid, 5% various vitamins, 0.5% phosphorus, 0.28% Fe, 0.041% Zn, 0.0035% Mn, 0.0023% Cu, 0.0012% Mg, and 0.0012% B; DELFANPLUS, a liquid fertilizer consisting of 24% free amino acids, 9% total nitrogen, 5% N-protein and 43% organic carbon. MACRO-MICRO mixture consisted of 5% N, 2.5% Zn, 2.5% Fe, 2% Mn, 0.5% Mg, and 0.5% Cu.

Plant sampling and measurements

French bean plants were grown for ten weeks, and various growth traits were measured during growth period, as well as at harvest time. Plant pods were harvested several times during their growth period. Cumulated yield of plants during 2-3 harvests (in each replicate) was recorded and the average was presented as plant final yield of fresh pods. Accordingly, total number of pods per plant was recorded. The average number of leaves and lateral shoots of plants were counted per replicate (plot) and calculated per plant. Chlorophyll index of leaves was recorded using a portable SPAD meter (model 502 Plus, Illinois, USA) with 10 reading per plant and at least 120 readings for each replicate, by which the average was recorded in the results. Plant stem diameter was measured by caliper (model Mitutoyo Japan). Average shoot dry weight per plant was calculated after drying four randomly chosen plants from each replicate at 65°C for 24 hours.

Total Soluble Solid (TSS) as Brix index was determined with a refractometer using a drop of pod extracted juice. For determination of pod pH and titratable acidity, 10 g of fresh pods (from each replicate) was cut, crushed and centrifuged at 9000 rpm. Ten ml of supernatant was titrated using 0.1 M NaOH until a final pH of 8.2, and the pod acidity based on citric acid in 100 g fresh pods was calculated using the following formula:

TA= 100 ×M×N×V/S×n

where TA is the amount of pod acidity (mg/100 g FW), M the molecular weight of dominant pod acid (citric acid= 64 g) n: valence of dominant acid, N: normality of NaOH, S: weight of pod sample (g) and V the volume of consumed NaOH.

For measurement of pod vitamin C, 10 g of fresh pods from final harvest was gently washed, cut in small pieces and then crushed in a mortar in presence of 10 ml of 2% metaphosphoric acid. The mixture was immediately centrifugated at 9000 rpm (Eppendorf Centrifuge 5810R, Hamburg, Germany) for 5 minutes at 4°C. The supernatant was used for titration by 2,6 dichloro indophenols, and the amount of vitamin C for 100 g fresh pod was calculated in relation to records of a standard curve of L-ascorbic acid concentration of 0, 25, 50, 100 and 200 mg L⁻¹. Determination of pod protein content was done using Coomassie Brilliant blue G250 dye according to Bradford's method (1976). The nutrient concentration of N, K and Fe were determined in plant leaves and green pods using Kjeldahl, flame photometer and atomic absorption spectrophotometer methods.

Statistical analysis

Data were analyzed using SPSS 16 and differences among treatments were determined at 5% level by Duncan's test. Graphs were prepared using EXCEL Microsoft.

3. Results

Plant vegetative growth

Vegetative growth characteristics of plants are presented in Table 2. Application of various fertilizers had significantly improved plant growth. All the most important growth factors were improved especially by foliar application of organic-based chelate fertilizers (Table 2). In particular, plant height was increased by application of three organic chelates, while there was no difference among control plants and those plants treated with soil applied NPK or by foliar application of macro-micro mixture. Stem diameter was significantly higher in plants that received foliar application of organic chelates (Table 2). Number of leaves was maximum in plants treated with Biomin and Humifolin; however they had no significant difference with DelfanPlus treated plants. The least number of leaves was in control plants. Number of lateral shoots was increased by foliar application of Biomin and Humifoline; however, there was no significant difference among all organic fertilizers. There was no significant improvement of lateral shoots by soil applied NPK or foliar application of macro-micro mixture (Table 2). Plants treated with DelfanPlus produced longest internodes that showed no difference with Biomin or Humifolin treated plants (Table 2). Application of all fertilizer treatments, except foliar application of macro-micro mixture, resulted in significantly higher SPAD values compared to control plants (Table 2). Determination of shoot dry weight revealed that plant growth and biomass production was significantly improved by foliar application of the

organic fertilizers, as well as by soil applied NPK treatment. On the other hand, the maximum shoot dry weight was recorded for Biomin treatment, which showed significant difference with control and all other fertilization treatments (Table 2).

Plant yield and nutrient status

Application of all fertilizers increased the pod yield compared to control plants (Table 3). Plants produced significantly higher yield when they received foliar application of organic chelate fertilizers (Table 3). Number of seeds per pod and number of pods per plant had a similar trend, in which foliar application of three organic chelate fertilizers recorded higher values. Regarding pod length, plants treated with foliar application of three organic chelates had the longest pods compared to all other treatments. All fertilizer treatments increased the pod dry weight. Plants treated with Biomin produced significantly higher pod dry weight (Table 3), although application of all the organic chelates significantly increased pod dry weight compared to soil applied NPK or foliar application of macro-micro mixture.

Nutrient profile of plant leaves and pods were significantly increased by foliar application of organic fertilizers, only for N and K, and also by NPK soil

Table 2 - Effects of various fertilization treatments on vegetative growth traits of French bean plants in a calcareous soil under field conditions

Treatments	Plant height (cm)	Stem diameter (cm)	Number of leaves	Number of lateral shoots	Length of internode (cm)	SPAD index	Shoot dry weight (g)
Control	26 b	0.62 b	14 c	4 b	5.6 b	36 b	7.2 d
NPK	31 ab	0.79 b	19 b	5 b	5.5 b	40 a	11.3 c
Biomin	39 a	1:05	24 a	8:00	6.7 ab	41 a	22.1 a
Humifolin	39 a	0.96 a	24 a	7:00	6.1 ab	41 a	15.6 b
DelfanPlus	38 a	0.98 a	22 ab	6 ab	7:02	42 a	17.5 b
Macro-micro mixture	29ab	0.67 b	17 bc	4 b	5.1 b	38 ab	8.5 d

Data are mean of four replicates. Comparison of means was done using Duncan's test at 5% level.

Table 3 - Effects of various fertilization treatments on yield and fruit characteristics of French bean plants grown in a calcareous soil under field conditions

Treatments	Plant pod yield (g)	Number of seeds/pod	Number of pods per plant	Pod length (cm)	Pod dry weight (g)	Pod pH	Pod TSS
Control	31.4 c	2.9 c	17 c	5.5 b	8.3 d	6.20 a	1.7 a
NPK	38.3 b	3.8 b	28 b	6.5 b	10.8 c	6.30 a	1.8 a
Biomin	59.1 a	5.1 a	49 a	9.1 a	21.1 a	6.21 a	2.4 a
Humifolin	53.5 a	4.7 a	43 a	9.3 a	16.2 b	6.22 a	2.1 a
Delfan plus	56.6 a	4.8 a	45 a	9.6 a	16.6 b	6.23 a	2.0 a
Macro-micro mixture	36.7 b	3.9 b	27 b	6.5 ab	11.4 c	6.24 a	2.1 a

Data are mean of four replicates. Comparison of means was done using Duncan's test at 5% level.

application (Table 4). Foliar application of Biomin and DelfanPlus resulted in significantly higher K and Fe concentration in leaves, and K concentration of pods, compared to Humifolin. The lowest nutrient concentrations were recorded in control and macro-micro mixture treatments.

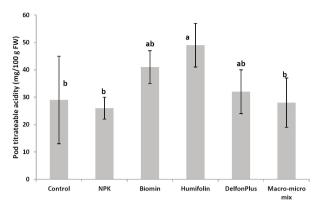
Determination of nutrients in green pods (Table 4) showed that the significant highest amount of N was in plants sprayed with Biomin aminochelate, and then by DelfanPlus, Humifolin and soil applied NPK, respectively. Potassium concentration was significantly higher in foliar spray of Biomin and DelfanPlus, while the lowest concentration was in pods treated with macro-micro mixture and in control plants. Iron concentration of green pods was significantly increased by organic fertilizers.

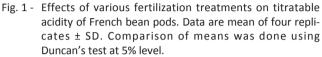
Pod quality

There was no significant difference in pod pH and pod total soluble solids (TSS) among treatments (Table 3). However, determination of titratable acidity (Fig. 1) revealed that plants treated with Humifolin resulted in significantly higher pod acidity (but not different from Biomin and DelfanPlus), while the least titratable acidity was measured in soil applied NPK, control and macro-micro treatments, that showed no difference with Biomin and DelfanPlus treatments (Fig. 1). Foliar application of three organic fertilizers resulted in significantly higher vitamin C content of pods compared to control plants (Fig. 2). Pod protein content was higher in plants treated with Humifolin and Biomin, and lower in control and plants treated by foliar application of macro-micro mixture. Pod protein content was intermediate for soil applied NPK and foliar application of DelfanPlus (Fig. 3).

4. Discussion and Conclusions

In the present study plant growth was improved by foliar application of three organic chelate fertilizers. Many parameters of vegetative growth as well as plant yield, nutrient content and fruit quality were improved by application of these three commercial





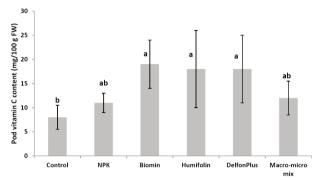
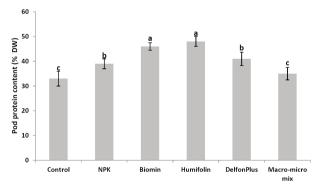


Fig. 2 - Effects of various fertilization treatments on vitamin C content of French bean pods. Data are mean of four replicates ± SD. Comparison of means was done using Duncan's test at 5% level.



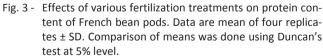


Table 4 - Effects of various fertilization treatments on some nutrients in leaves and pods of French bean grown in field with calcareous soil conditions

Treatments		Leaf			Pod			
Treatments	N (%) K (%) Fe (mg kg ⁻¹ DV		Fe (mg kg ⁻¹ DW)	N (%)	Fe (mg kg ⁻¹ DW)			
Control	2.1 c	1.7 c	55.3 c	3.7 c	2.8 c	77.2 b		
NPK	2.7 b	2.6 a	64.1 c	5.2 b	4.6 b	85.1 b		
Biomin	3.5 a	2.8 a	126.3 a	6.5 a	5.3 a	108.3 a		
Humifolin	2.9 b	2.3 b	97.7 b	5.6 b	4.7 b	96.6 a		
DelfanPlus	3.2 ab	2.6 a	118.5 a	5.7 b	5.1 a	99.0 a		
Macro-micro mixture	2.3 c	1.8 c	66.1 c	3.7 c	2.7 с	82.4 b		

Data are mean of four replicates. Comparison of means was done using Duncan's test at 5% level.

organic fertilizers. Increasing in plant growth and various yield traits were also reported in other studies (Machado et al., 2008; Garcia et al., 2011; Naseri et al., 2013; Salwa, 2011; Ghasemi et al., 2014; Sadak et al., 2015; Souri and Yarahmadi, 2016). In a recent study it was shown that foliar application and to lesser extent soil application of a commercial aminochelate fertilizer increased the growth, yield and quality of tomato, cucumber and bean plants (Souri et al., 2017). Foliar application of a mixture of amino acid on bean plants significantly improved the tolerance to seawater salinity stress (Sadak et al., 2015). Similarly, application of amino acids significantly improved growth parameters of shoots and fresh weight as well as pod yield of soybean plants under pathogen infection (Saeed et al., 2005). Vegetative growth of plant height and dry weight of potato plants were increased by foliar application of amino acids (El-Zohiri and Asfour, 2009). Three foliar application at 6-7 leaves stage and two more sprays in two weeks intervals using Fe and Zn aminochelates with different concentrations up to 0.3%, resulted in 15-35% higher potato tuber yield per hectare (Pourebrahimi et al., 2013).

Stimulatory effects of amino acids on plant growth characteristics have been well documented, particularly under adverse climatic conditions such as salt and drought stresses (Rai, 2002; Zhou et al., 2007; Garcia et al., 2011; Salwa, 2011; Sadak et al., 2015). Amino acids are key important player in plant metabolism. They are the intermediate compounds in nitrogen assimilation, and represent the main form by which nitrogen is translocated within the plant through phloem (Marschner, 2011). Various amino acids and peptides are precursor of physiologically important phytohormones (Cobbett and Goldsbrough, 2002; Marschner, 2011) or they are involved in detoxification of different toxins within the plant cells (Cobbett and Goldsbrough, 2002; Souri, 2015). In addition to the amino and carboxyl groups, amino acids have a side chain or R group that is attached to the α -carbon. Each amino acid has unique characteristics arising from the size, shape, solubility and ionization properties of its R group. Nevertheless, the side chain of amino acids exerts a deep effect on their biological activity as well as on the structure and activity of proteins. By far, glycine is the main and widespread used amino acid in manufacturing aminochelate fertilizers, despite frequently a mixture of amino acids may be included (Souri, 2015). Similarly, the stimulating effect of foliar or soil application of humic acid on increased plant growth

and nutrient uptake has been shown (David *et al.*, 1994; El-Ghamry *et al.*, 2009; Salwa, 2011; Canellas *et al.*, 2015). The biostimulant effects of humic substances are characterized by both structural and physiological changes in roots and shoots related to nutrient uptake, assimilation and distribution (nutrient use efficiency traits). In addition, they can induce shifts in plant primary and secondary metabolism related to abiotic stress tolerance which collectively modulate plant growth as well as promoting fitness (Canellas *et al.*, 2015).

In the present study, improved growth and plant performance of French bean might be also due to the higher nitrogen and micronutrients content of plant leaves. The N, K and Fe concentrations in plant leaves were significantly improved by foliar applications of organic chelate fertilizers. Metal ions such as Fe, Zn, Mn and Cu are essential for healthy plant growth, being required for various metabolism reactions (Marschner, 2011). They have direct and distinct effects on plant performance, as well as on yield and quality parameters. However, uptake of micronutrients such as iron by roots from soil could be limited due to low chemical stability and precipitation of these elements particularly in calcareous soil (Fernández and Ebert, 2005; Souri, 2015). In calcareous soils, with high pH and carbonate-bicarbonates levels, plants are prone to iron and other micronutrient deficiencies. Lime-induced chlorosis is one of the most important nutritional disorders, affecting many plant yield and quality traits.

Aminochelate fertilizers represent an excellent N source for plant, in both foliar and soil applications. In present study, N concentration of leaves and pods were significantly improved by organic fertilizers. Higher nitrogen content of plant was also reported for radishes (Liu *et al.*, 2008) and marigold (Souri and Yarahmadi, 2016), when plants were treated by foliar application of aminochelate fertilizers. Improvement in nutrient elements profile of tomato was observed when amino acids were applied in nutrient solution, which finally improved plant growth and nutrient concentrations, particularly N status of plants (Garcia *et al.*, 2011).

Nitrogen has an important role in growth, yield and quality of crops and must be usually applied to meet the plant needs. French bean can fix atmospheric N2, so it may need less N fertilization; however, in some parts of Iran farmers use also high rates of urea to enhance plant growth. On the other hand, application of high amount of N fertilizers could lead to significant reduction in yield and quality, as well as various pollutions. Leaf greenness, as the best health indicator of plants, depends on chlorophyll biosynthesis and concentration, which in part is affected by N and micronutrients. Aminochelates generally contain all these effective nutrient elements. Application of aminochelates, as a source of N fertilizer, can also result in lower nitrate accumulation in plant tissues. High nitrate content of plant tissues is a negative factor, particularly in leafy vegetables that are fresh consumed. Application of reduced form of nitrogen (such as ammonium or amino acids) instead of oxidized form (nitrate) can lead to less nitrate accumulation in plant tissues (Marschner, 2011; Souri et al., 2017). On the other hand, plant might have different response to various fertilizers of a given nutrient (Souri, 2015). However, there are always distinct clear responses of vegetative and reproductive growth, as well as quality parameters to nitrogen fertilizers (Marschner, 2011; Souri, 2016).

The higher efficiency of aminochelate fertilizers can be due to the chelating effects of amino acids or organic acids on nutrient elements. The chelating effects of amino acids on nutrients has been commercially used for improving nutritional status of animals and human for more than 6 decades, and for plants in recent years (Souri, 2016). As it is well known, amino acids are "zwitterions" in biological systems, and have distinct different behavior in acidic and basic solutions, to maintain the pH of the system. The structure of an amino acid allows it to act both as an acid and a base, depending on solution pH. This behavior is quite important in plant nutrition, as pH is one of the main factors responsible for nutrient use efficiency, and frequently high pH (in calcareous soils) and low pH (in acidic soils) restrict nutrients solubility and bioavailability (Souri, 2015). Nevertheless, in present study organic chelate fertilizers had various composition of one or several components of amino acid or nutrients. Conducting scientific research with such amino or organic chelates due to their mix nature of various components is quite difficult (Souri, 2016).

In conclusion, foliar application of organic chelate fertilizers resulted in higher plant growth under calcareous soil conditions. Aminochelates are composite fertilizers of amino acids and various nutrient elements particularly iron and zinc and separating the effects of each component is relatively difficult. The effect of organic chelate fertilizers particularly aminochelates on many physiological and molecular responses of plants has not been well studied. Application of organic chelate fertilizers can avoid all negative effects of routine chemical salt fertilizers such as urea or ammonium nitrate, including leaching, volatilization and nitrate accumulation in vegetable tissues. Therefore, they represent modern and suitable alternatives to simple salts, and even to commercial synthetic chelates such as EDTA due to their cheaper price. Nevertheless, these claims need to be evaluated in deep in future studies.

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