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Benefits of organic fertilizer spray on growth quality of chili pepper seedlings under cool temperature

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Abstract

Production of healthy, vigorous seedlings is necessary for optimum growth and high yields in field and greenhouse production of peppers. Pepper (*Capsicum annuum*) is a warm season crop and applying cooler temperatures during seedling growth may have some benefits, but it can reduce plant growth. In present study, effects of foliar application of synthetic and organic fertilizers were evaluated on growth and quality of chili pepper seedlings under greenhouse conditions with cool temperature of 15±3ºC. Treatments were foliar application of fertilizers in a 0.2 % concentration of: 1) N20:P10:K20, 2) Biomin (an organic aminochelate fertilizer), 3) Humifolin (a humic acid based fertilizer), 4) Biomin+Humifolin, 5) a synthetic macro-micro mixture, 6) soil application of NPK (600 $mg.kg^{-1}$ of the N20:P10:K20 formulation) and 7) a no fertilizer control. Fertilizer treatments improved seedling overall growth except for plant height and stem diameter. Most growth and quality traits were best improved by foliar application of organic Biomin aminochelate followed by Humifolin fertilizer. Higher values for leaf area, leaf number, chlorophyll index, root and shoot biomass, and leaf concentration of soluble sugars, N, K, Ca and Zn, were attributed to foliar application of Biomin aminochelate and Humifolin. The mixture of Biomin+Humifolin had reduced values of those parameters indicating possible negative interaction when these two organic fertilizers are mixed. So, under cool temperature foliar feeding of organic fertilizers (aminochelate) can effectively improve nutrients status and transplant's quality of pepper.

Key words: Aminochelate, *Capsicum annuum*, humic acid, nutrient elements, transplants

Introduction

Production of healthy, vigorous seedlings is necessary for the optimum growth and high yields in field and greenhouse production of peppers. Nursery production of vegetable seedlings is an economical and specialized industry (Dufault, 1998). However, in many parts of the world, particularly in developing countries, farmers still produce their own transplants.

Plant nutrition plays important role in growth and quality of seedlings (Marschner, 2011; Masson *et al*., 1991), and can be used for manipulating transplant quality and growth (De Grazia *et al*., 2008; Melton and Dufault, 1991; Weston and Zandstra, 1989). The role of each nutrient in plant physiology are well known, and it is possible to control seedling growth by manipulating their concentrations and ratios. Various nutrient elements can affect the general seedling health and development (Dufault, 1998). A shortage in essential nutrient elements generally leads to reduced seedling growth and less tolerance to biotic and abiotic stresses. Proper application of fertilizers can improve seedling growth and quality. Nitrogen generally is the most important nutrient in plant growth and physiology (Marschner, 2011), and its deficiency can result in reduced growth and yield, as well as early plant senescence (Marschner, 2011; Souri, 2016). Many plant growth parameters are improved by application of nitrogen fertilizers. Mixed fertilizers such as NPK generally result in better and balanced nutrition that can improve plant growth and produce harder seedling for shipment to remote areas (Melton and Dufault, 1991). However, higher amounts particularly of nitrogen can significantly hamper seedling quality and plant production.

Routine chemical fertilizers, their amounts, and method of application can lead to contamination of water, soil, air and food, since not all of the material is used by the plant and is released to the environment. New approaches for effective formulations toward higher efficiency are required. Foliar application represents an alternative to soil application of fertilizers (Dehnavard *et al*., 2017), in which it is important to choose the right source and concentration of the material applied (Fageria *et al*., 2009; Souri, 2016). This can lead to improved effectiveness and less damage to leaves. In seedling transplant production under specific conditions, foliar application can represent an effective method of nutrient delivery to foliage for optimum plant growth.

Organic fertilizers represent a source of nutrients for plants that is an alternative to synthetic fertilizers. In addition to their nutrient content, they contain bioactive molecules with generally beneficial effects on plant growth and productivity (Fahimi *et al*., 2016; Souri, 2016). Aminochelate fertilizers have effective formulations and various advantages (Souri, 2016; Sánchez-Sánchez *et al*., 2005). They are almost new and among the most effective fertilizers which are highly acceptable by farmers (Souri, 2016). Their application on crops gives better plant growth and productivity compared to routine chemical fertilizers (Garcia *et al*., 2011; Fahimi *et al*., 2016; Souri and Yarahmadi, 2016; Cerdan *et al*., 2013). However, there is little information regarding seedling and transplant responses to aminochelate fertilizers. On the other hand, to optimize plant nutrition in cropping systems, effective management strategies and new approaches are required. Under cool temperature uptake of nutrients by roots of chili pepper is hindered, and this may be mitigated by foliar spray of organic fertilizers. Therefore, in present study foliar application of organic fertilizers were evaluated and compared to soil and foliar application of synthetic fertilizer on seedling growth of chili pepper (*Capsicum annuum* var annuum).

Material and methods

The study was in a controlled environment greenhouse during March and April 2014. The experiment was arranged in a completely randomized design with 7 treatments and 8 replications. Black plastic pots (13 cm height and 8 cm diameter) were filled with 1 kg mixture of 1:1 sand and field soil (silty-loam), for which the nutrient load had been determined (Table 1). Ten seed of pepper were sown in each pot. One week after germination, plants were thinned to 6 and 1 week later to 3 plants per pot. Different fertilizing treatments were applied as follows: control (spray of only distilled water), soil application of N20:P10:K20, foliar application of N20:P10:K20, foliar application of Biomin (aminochelate), foliar application of Humifolin, (humic acid-based organic fertilizer), foliar application of Biomin+Humifolin mixture, and foliar application of a hand-made micro-macro mixture.

The experiment was begun on 1 March and continued until end of April 2014, with greenhouse conditions of 15±3 °C and 70±5 % humidity. Foliar spray (0.2 %) of all materials was performed in 5 applications, started two weeks after emergence. Application was done in the morning (1 hr after sunrise) using a portable spryer, with the material directed to the upper and the lower leaf surface. The first application was 2 weeks after emergence and the remaining applications at 1 week intervals.

The elemental composition of fertilizers was as follow: Biomin, a liquid, light brown material containing 2 % N, 2.5 % Zn, 1.5 % Mn, 1 % Fe, 0.4 % Mg, 0.4 % Cu, and 0.02 % Mo; Humifolin; a liquid, dark material containing 42 % organic compounds including 37 % fulvic and humic acids, 7 % vitamins, 0.5 % P, 0.28 % Fe, 0.041 % Zn, 0.0035 % Mn, 0.0023 % Cu, 0.0012 % Mg, and 0.0012 % B. The macro-micro nutrient mix was prepared as follow: 5 % N, 2.5 % Zn, 2.5 % Fe, 2 % Mn, 0.5 % Mg, and 0.5 % Cu suspended in distilled water. In N20:P10:K20 treatment, 2 applications from 10 g L^{-1} solution in a final amount of 600 $mg.kg^{-1}$ soil were applied. The first half was uniformly mixed into the soil before seeding, and the remaining half was applied to pots 1 week after emergence.

Due to soil culture and low temperature experimental conditions, seedlings were harvested 7 weeks after emergence. The growth and quality factors *viz*., plant height, number of leaves, leaf area (using leaf area meter Model CI 202, Washington, USA), chlorophyll index (using SPAD meter model 502 Plus, Illinois, USA), and stem diameter (using Kolis, Mitutoyo, Japan) were recorded 1 week before harvest. At harvest fresh weight of seedlings was determined and dry weights calculated after plants were dried for 48 h in a forced air (65 ºC) oven. Soluble carbohydrates of leaf were measured using ninhydrin method, in which 0.1 g of leaf fresh tissues was extracted in 2.5 mL ethanol 80 % at 95°C for 60 min. The extract then filtered and the alcohol removed by evaporation. The anthrone reagent was used for preparation of samples and absorption measured at 625 nm. A standard glucose curve was also used for calculation of carbohydrate content. Nitrogen concentration of leaves was determined by the Kjeldahl method, K using flame photometry, and Ca and Zn using atomic absorption spectrophotometry.

Data were analyzed with SPSS version 16.0 (SPSS, Inc., Chicago IL). Comparison of means was with Duncan's multiple range test.

Results and discussion

The results of soil analysis showed that soil has silty-loamy texture with rather low content of organic matter, nitrogen and potassium (Table 1). There were treatment effects for chlorophyll index, leaf area, root fresh weight, number of leaves and shoot fresh weight, shoot dry weight, leaf sugar content, and leaf N, K, Ca and Zn but not plant height (9.3-10.5 cm) and stem diameter (1.9-2.2 mm) (Table 2).

The most leaves were formed due to treatment with Biomin and Humifolin, which was greater than for control plants (Table 3). Average single leaf area was highest in plants treated with Biomin+Humifolin, and with Biomin and Humifolin alone. The lowest leaf area was in the control. The highest chlorophyll index was in Biomin and then Humifolin treated plants (Table 3). The lowest leaf chlorophyll index was in the control plants and plants treated with foliar applications of NPK (Table 3). Foliar spray of Biomin resulted in the highest plant root fresh weight, while the lowest values were in control plants and those plants treated with the macro-micro mix (Table 3). Plant shoot fresh weight was significantly higher in Biomin, Humifolin, soil NPK or Biomin+Humifolin treatments versus other treatments. The lowest shoot fresh weight was in control plants and plants treated with foliar NPK (Table 3). The highest shoot dry weight was in Biomin treated plants, that was similar to plants treated with Humifolin, Biomin+Humifolin, and macro-micro treatments. The lowest shoot dry weight was in control plants (Table 3).

Table 1. Physico-chemical properties of the soil

Soil texture	Sand (%)	Silt (%)	Clay (%)	EC[a] (ds/m)	pH	Organic carbon	Nitrogen %	Phosphorus ($mg.kg^{-1}$)	Potassium ($mg.kg^{-1}$)
Silty-loamy	31	49	20	0.915	7.17	0.62	0.096	15.2	256

[a] EC = electrical conductivity

Table 2. Analysis of variance for growth related traits of pepper seedlings

Source	df	Plant height	Stem diameter	No. of leaves	SPAD value	Leaf area	Root fresh weight	Shoot fresh weight	Shoot dry weight	Leaf sugar	Leaf N	Leaf K	Leaf Ca	Leaf Zn
Treatment	6	1.90ns	0.068ns	3.04*	50.89**	4.58**	0.005**	0.273*	0.004**	11.59**	0.66**	0.48**	0.14**	215.6**
Error	49	1.024	0.041	0.964	3.628	0.847	0.001	0.058	0.001	0.305	0.050	0.037	0.025	11.276
Total	56													

ns, *, ** not significant, significant at 5 % or 1 %, respectively.

Table 3. Comparison of means for some morphophysiological traits of chili pepper seedlings under different fertilization treatments.

Treatment	Plant height (cm)[x]	Stem diameter (mm)	Number of leaves	Leaf area (cm^2)	Chlorophyll index	Root fresh weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
Control	9.3±0.9[a]	1.9±0.18[a]	7.9±0.6[b]	9.0±0.8[c]	28.1±1.6[b]	0.19±0.03[b]	2. 6±0.2[b]	0.19±0.02[b]
Soil NPK	9.9±1.1[a]	2.2±0.23[a]	8.6±0.7[ab]	11.6±0.9[b]	30.2±1.4[ab]	0.21±0.03[ab]	3.0±0.3[a]	0.20±0.03[b]
Foliar NPK	9.4±1.4[a]	2.1±0.22[a]	8.5±1.2[ab]	11.2±1.1[b]	29.1±1.4[b]	0.20±0.02[ab]	2.7±0.2[b]	0.20±0.03[b]
Biomin	10.3±0.5[a]	2.2±0.17[a]	9.7±0.9[a]	12.2±0.9[ab]	34. 5±2.3[a]	0.25±0.03[a]	3.1±0.2[a]	0.25±0.02[a]
Humifolin	10.5±1.2[a]	2.1±0.19[a]	9.4±1.3[a]	12.3±1.1[ab]	33.5±2.4[a]	0.23±0.03[ab]	3.0±0.3[a]	0.23±0.03[ab]
Bio+Humi[y]	9.8±0.9[a]	2.1±0.26[a]	8.4±0.8[ab]	13.8±0.8[a]	30.6±2.0[ab]	0.23±0.03[ab]	3.0±0.2[a]	0.23±0.04[ab]
Macro-Micro	10.5±0.9[a]	2.1±0.11[a]	8.3±1.1[ab]	10.5±0.7[b]	31.8±1.9[ab]	0.20±0.02[b]	2.8±0.2[ab]	0.22±0.02[ab]

All fertilizers were foliar sprayed, and NPK was used as both soil and foliar application.
[x] values in columns followed by the same letter are not significantly different at DMRT (*P*=0.05).
[y] Bio+Humi = Biomin+Humifolin

Soil application of NPK, and plants treated with foliar application of Biomin, Humifolin or Biomin+Humifolin had higher amounts of soluble carbohydrates compared to other treatments (Table 4). The least carbohydrate content was for control plants. Foliar application of Biomin, soil application of NPK and foliar application of Biomin+Humifolin improved N concentration of leaves compared to control (Table 4). The highest N concentration was for plants treated with Biomin. Leaf K concentration (Table 4) was highest in plants treated with soil application of NPK. Leaf K concentration was also improved in response to organic fertilizer applications compared to control. The lowest leaf N and K concentrations were in control plants.

The highest Ca concentration was in plants treated with Humifolin, Biomin and soil applied NPK treatments (Table 4). The lowest Ca concentration was in leaves of plants treated with foliar applications of NPK. All fertilizer treatments improved leaf Zn concentration (Table 4) compared to control. Foliar application of Biomin, soil applied NPK, Humifolin and Biomin+Humifolin produced the highest leaf Zn concentration. Foliar application of the macro-micro mix had higher Zn in leaves compared to controls (Table 4).

Although soil application of organic fertilizers was not included in the study, their foliar applications resulted in improved records compared to soil or foliar application of NPK. Biomin (an aminochelate fertilizer), based on its composition classified as a complete fertilizer can supply a range of nutrients to plants. It has been determined that amino acids can improve plant growth and overall tolerance to stresses (Marschner, 2011).

Better growth and performance of seedlings occurs with application of nutrition through roots (Dufault, 1986; Melton and Dufault, 1991; Weston and Zandstra, 1989), or through foliage (Dehnavard *et al*., 2017; Fageria *et al*., 2009). Increasing nitrogen level generally improves plant vegetative development (Melton and Dufault, 1991). Under stress, seedlings may require higher nutrient concentrations or simplified delivery techniques (Souri, 2016). In this study, foliar application of Biomin and Humifolin improved leaf concentrations of N, K, Mg, Ca and Zn. This could be due to growth promoting effect of these fertilizers and their possible semi-hormonal effects (Fahimi *et al*., 2016). Their nutrient content is also a factor affecting growth. Increasing Mn, Fe, Zn and Cu supply level could improve plant growth and tissue concentrations of N, P and protein as well (Elabdeen and Metwally, 1982; Souri, 2016).

A main factor in seedling quality is concentration of leaf chlorophyll and soluble carbohydrates. The period of seedling production, light intensity and amount of nutrients affect leaf chlorophyll index and soluble sugars (Corut *et al*., 1993). Supplying seedlings with essential nutrient elements could improve leaf greenness and sugar status (Default, 1998; Melton and Dufault, 1991). With inadequate nutrition, there may be additional time required to reach optimum leaf sugars, which could affect time to harvest.

Amino and organic acids are efficient natural chelators (Souri, 2016; Zhou *et al*., 2007). These compounds are used for commercial synthesis of organic fertilizers. In the present study, organic fertilizers contained 5-25 % amino acid which improved growth and quality of pepper seedlings. In addition, nitrogen from amino acids has a role in general seedling tolerance to stress (Cerdán *et al*., 2013), as was the case in this study.

Foliar or root feeding of amino acids containing Fe, when plants

Table 4. Comparison of means for leaf soluble carbohydrates and some nutrient elements of chili pepper seedlings under different fertilization treatments

Treatment	Leaf soluble carbohydrates (mg g^{-1} DW)	Leaf N content (% DW)	Leaf K content (% DW)	Leaf Ca content (% DW)	Leaf Zn content (mg kg^{-1} DW)
Control	4.3c[x]	1.56c	1.75c	1.09ab	33.9c
Soil NPK	6.7a	2.14ab	2.51a	1.25a	41.1ab
Foliar NPK	5.2b	1.9b	1.99b	0.98b	33.8c
Biomin	7.1a	2.51a	2.02b	1.28a	47.4a
Humifolin	6.4a	1.9b	1.95bc	1.36a	42.4ab
Bio+Humi[y]	7.2a	2.03ab	1.90bc	1.14ab	45.2ab
Macro-Micro	4.6bc	1.90b	1.84bc	1.08ab	39.9b

All fertilizers were foliar sprayed, and NPK was used as both soil and foliar application.
[x] values in columns followed by the same letter are not significantly different at the 5 % Duncan's test.
[y] Bio+Humi = Biomin+Humifolin

were growing in nutrient solution with high alkalinity conditions, resulted in better tomato growth through higher chlorophyll index and improved vegetative traits (Cerdán *et al*., 2013). However, plants may have different tolerance to specific amino or organic acids. Application of 0.2 and 0.7 mL L^{-1} of amino acids for root and foliar feeding can be toxic for seedlings (Cerdán *et al*., 2013). When 50, 100, 150 and 200 mL L^{-1} humic acid were applied to tomato roots and egg-plant (*Solanum melongena* L) seedlings the highest nutrient content in tomato was with 100 mL L^{-1} and for egg-plant at 200 mL L^{-1}, indicating species differences in response to organic fertilizers (Dursun *et al*., 2002). Leaf chlorosis and necrosis in mature cucumbers was induced by 0.1 % and 0.2 % Biomin (Fahimi *et al*., 2016). It has been demonstrated that application of Fe-EDDHA together with a mixture of amino acids could improve uptake and concentration of Fe in tomato (Sánchez-Sánchez *et al*., 2002; 2005).

Transplant production is a specialized industry which aims to produce vigorous seedlings with short internodes, large root systems, adequate leaf area and high carbohydrate and chlorophyll content without deficiency symptoms. This quality is generally achieved by application of routine fertilizers, and often by application of growth retardants, which could have negative effects on plant growth parameters (Aloni and Pashkar, 1987).

In present study, foliar feeding of pepper seedlings using organic fertilizers demonstrated promising effects on seedling growth and quality. While seedling height was not increased by foliar organic fertilizers treatment, but higher biomass, chlorophyll and sugar concentrations indicate improved seedling quality. Application of organic fertilizers based on amino or organic acids play a role in nutrition management of cropping systems. In addition to micronutrients, macronutrients could be applied to plants in organic fertilizers. Application of organic fertilizers such as aminochelates could be suitable alternatives for synthetic fertilizers for high quality seedling production with less environmental pollution and can also reduce transplant growth period.

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