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Effect of Ni on yield, quality and N assimilation of cucumber (*Cucumis sativus* L.) grown with urea or nitrate

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Abstract

The effects of Ni concentrations in the nutrient solution on the yield, quality and N assimilation of cucumber plants were evaluated in plants grown either with urea or nitrate as the sole N source. The cucumber plants (*Cucumis sativus* cv RS189 and Vikima) were treated with two N sources, urea and nitrate as $NaNO_3$ at 200 mg L^{-1}, and three concentration of Ni as $NiSO_4.6H_2O$ (0, 0.5, and 1 mg L^{-1}). Treatments were arranged in a randomized block design with six replicates. The highest concentration of Ni in the leaves (1.2 mg kg^{-1} DW) was observed in the urea-fed plants at 1 mg L^{-1} Ni concentration. Addition of Ni up to 0.5 mg L^{-1} had no effect on the fruit Ni concentration in the both urea and nitrate-fed plants. Ni supplement (0.5 mg L^{-1}) increased the yield significantly (10 and 15% in RS189 and Vikima, respectively), in urea-fed plants but decreased when 1 mg L^{-1} Ni applied to the solutions. Nitrate-fed plants had higher percentage of total soluble solids compared to urea-fed plants. Nitrate concentration of the fruits in urea-fed plants in both cultivars was approximately 50% less than those nitrate-fed plants. The reduction of nitrate concentration in the fruits became more pronounced as the Ni concentration increased in the solution. The rate of photosynthesis (Pn) in urea-fed plants continuously increased with the increase of the Ni concentration in the solution. Both N concentration and NR (Nitrate Reductase) activity of young leaves were higher in urea-fed plants at 0.5 mg L^{-1} Ni concentration. Ni supplements enhanced the growth and yield of urea-fed plants by the increase of Pn, N concentration and NR activity. It can be concluded that Ni supplements (0.5 mg L^{-1}) improves yield, quality and NR activity in urea-fed cucumber plants.

Key words: Ni, cucumber, N, yield, quality, urea, nitrate

Introduction

Nickel (Ni) is an essential nutrient for higher plants because of its role as a component of urease enzyme (Dixon *et al.*, 1975 and Brown *et al.*, 1987). This element is required for N metabolism where N is applied in the form of urea (Eskew *et al.*, 1984). The deficiency of Ni depresses urease enzyme activity (Eskew *et al.*, 1983) and other enzymes responsible for the nitrate reduction (Brown *et al.*, 1990), consequently reducing synthesis of protein and N compounds (Brown *et al.*, 1990). This lead to the accumulation of urea, nitrate, and certain amino acids resulting in the incidence of leaf chlorosis and meristem necrosis of cucumber plants (Watanabe and Shimada, 1990). Therefore, low level of Ni is crucially important for the achievement of optimal yield of commercial crops (Eskew *et al.*, 1983, 1984). However, it appears that there is a difference among cucumber cultivars in response to Ni supplements.

Terrestrial plants are able to uptake different forms of N including nitrate, ammonium and urea. Urea is an important source of N fertilizer which is applied in soil and soilless culture systems. In hydroponic cultures urea can replace those nitrate N fertilizers provided that the adverse effects of urea accumulation on plant growth can be overcome (Zhu *et al.*, 1997). The main problem which is associated with urea use for higher plant nutrition is the unavailability of urea for the plant metabolism unless hydrolyzed to ammonium and carbon dioxide by urease (Marschner, 1995; Watanabe and Shimada, 1990).

Many studies showed that Ni is a part of the active centre of urease (Dixon *et al.*, 1975) and that activation decrease depends upon Ni in the plants (Marschner, 1995). A critical Ni concentration in the leaf of the plants could depend upon employing the N source; therefore different Ni concentrations might be required for the achievement of optimal plant growth. In this study, the effects of Ni concentration in the solution on the N assimilation, yield and fruit quality of cucumber plants grown in hydroponic supplied either with urea or nitrate was investigated.

Materials and methods

Plant growth conditions and treatments: Seeds of cucumber (*Cucumis sativus* cv. RS189 and Vikima) were sown in the propagation cubes. When two leaves fully expanded, four plants were planted in the bags (each bag was 100×20×10cm) filled with mix of perlite and vermiculite (1:1). Six bags were laid out on the floor in six rows with 1.2 m between rows. Each bag was considered as a plot and each row as block (3.3 plants per m^2). The first and last slabs on the rows were considered as guard rows. The treatments were randomized within rows to give a randomized complete block design with six replicates.

The greenhouse was under natural sunlight during spring and summer and the temperature was set 28±3 and 20±3 ^oC in day and night, respectively. The basic nutrient solution was prepared based on Xue *et al.* (2000) containing K_2SO_4: 2, $CaCl_2.2H_2O$: 1.5, $MgSO_4.7H_2O$: 1 and $NaH_2PO_4.2H_2O$: 2.3 mM and all micronutrients in half strength of Hoagland's solution. The concentration of N in all nutrient solutions was kept at 200 mg L^{-1} by adding either urea or NO_3 as $NaNO_3$. Three concentrations of Ni as $NiSO_4.6H_2O$ (0, 0.5 and 1 mg L^{-1}) were applied. The solution pH was adjusted to 6.5 by adding H_2SO_4. The bags were

equipped with drippers (4 L h^{-1}) which enabled to supply nutrient solutions with different solutions to each bag. The drippers were placed at the base of each plant and a timer was used to ensure that all plants receive an equal volume of solutions; an excess of 20% solution was applied to minimize EC and pH changes inside the bags.

Data collection and chemical analysis: The fruits (50-60 g) were harvested twice per week from the beginning of June until the end of September for 16 weeks and the fresh weight of the fruits was recorded. At the end of experiment, all plants from each treatment were taken to measure leaf area and weight. The leaf area was measured using leaf areameter (Li-Cor, model Li -1300, USA). After weighing the leaves they were dried at 80°C in an air forced oven for 48 h.

Fruit quality was measured in a representative sample collected at the same position from plants in each treatment. Total soluble solids (TSS) were measured in undiluted juice with a hand-held refractometer. A thin layer of the middle of fruit skin (0.5 mm), was removed by a sharp razor and fruit color or the extent of greening was measured using a chlorophyllmeter (SPAD-502, Minolta, Japan). The concentrations of N and Ni in the youngest fully expanded leaves were determined by Kjeldal method and atomic absorption spectrophotomtry, respectively (Perkin Elmer, Model 110, USA).

Photosynthetic rates of the mid-lamina portion of the youngest fully expanded leaves of two plants from each treatment were measured using a portable photosynthesis meter (Walz, Model Da1010, Germany). The flow rate and PAR were set to 800 min and 1500 µmol $m^{-2}s^{-1}$, respectively. Single measurement was carried out between 9:00 and 14:00 O'clock.

Nitrate reductase (NR) was measured in the young leaves (third or forth from top) according to Klepper *et al.* (1971). The leaf tissue (0.2 g fresh weight) was placed in reaction mixture containing 0.1 M potassium phosphate buffer (pH 7.5), 0.02 M KNO_3, 50% isopropanol, 0.05 chloramphenicol at 30°C for 1 h in the dark. The indicative Grease reagent containing 0.001 g 1-naphtyl-ethylene diamine, 0.01 g suphanilic acid, and 0.9 g tartaric acid was added to each sample. The concentration of nitrite formed during the reaction was measured spectrophotometrically.

Nitrate concentration in the dried fruits and leaves was determined according to Cataldo *et al.* (1975). Approximately 0.2 g of dried tissue powder was placed in 125 ml container and 25 ml hot water was added. The samples were shaken for 30 min on a Wristaction shaker and filtered through Whatman No 42 filter paper. Nitrate in the filtered solution was determined by adding a 0.2 ml sample aliquot to 0.8 ml of 5% (w/v) salicylic acid (dissolved in H_2SO_4) and 19 ml 2 N NaOH. Samples were allowed to cool at room temperature for 1 h, and developing color was measured at 410 nm by spectrophotometer (Motic, CL-45240-00, China).

A statistical analysis was done using analysis of variance in the SAS 8.2 software and the means were separated by LSD test (*P*=0.05). The graphs were drawn in Excel software.

Results

Ni concentration of leaves and fruit in both urea and nitrate-fed plants increased significantly with the increase of the Ni concentration in the solutions (Table 1). In the leaves, the higher concentration of Ni was observed in the 1 mg L^{-1} Ni concentration in both cultivars. Like leaves, addition of Ni to the solution increased the Ni concentration in the fruits. However, no significant difference was found between 0.5 and 1 mg L^{-1} Ni concentration. In this experiment, the N source had no significant effect on the Ni concentration in the leaves. No visual symptom of urea toxicity was observed in the cucumber plants at various rates of Ni.

Table 1. Influence of N source and Ni concentraion on the concentration of Ni in the leaves and fruits

Treatments		Ni concentration in leaves (mg kg^{-1} DW)		Ni concentration in fruits (mg kg^{-1} DW)	
		RS189	Vikima	RS189	Vikima
Ni_0	Nitrate	0.07b	0.06b	0.04b	0.03b
$Ni_{0.5}$		0.37a	0.39a	0.06b	0.04b
Ni_1		1.19a	1.35a	0.18a	0.13a
Ni_0	Urea	0.06b	0.07b	0.02b	0.03b
$Ni_{0.5}$		0.23a	0.25a	0.03b	0.04b
Ni_1		1.20a	1.30a	0.08a	0.10a
F values					
Ni×N		1.30*	1.70*	0.0 7 ns	0.08 ns
N source		2.70**	2.90**	1.06 *	1.35 *
Ni		1.80 ns	0.82	0.05 ns	0.02 ns

*, ** significant at *P*=0.05, *P*=0.01, respectively; ns- non significant

Either N source or Ni concentration had no significant effect on the fresh weight of leaves, however dry weight of RS 189 in urea-fed plants was higher than that with nitrate-fed plants (Table 2). In general, leaf area was not affected by N form supplements however, in both cultivars the highest leaf area was found in urea-fed with Ni at 0.5 mg L^{-1} concentration (Table 2). The yield of cucumber significantly increased in urea-fed plants (Table 2). The highest yield in urea-fed plants, 2.1 and 1.8 kg $plant^{-1}$ was obtained from RS189 and Vikima, respectively. Ni supplement (0.5 mg L^{-1}) increased the yield significantly (10 and 15% in RS189 and Vikima, respectively), but decreased when 1 mg L^{-1}Ni applied to the nutrient solution in urea-fed plants.

The effect of Ni concentration and N source on fruit quality is given in Table 3. Dry matter and colour of fruit were not affected by the treatments. TSS in the plants with nitrate nutrition was higher than that with urea nutrition. Nitrate concentration of the fruits in urea-fed plants in both cultivars was approximately 50% less than those nitrate-fed plants (Table 3). The reduction of nitrate concentration in the fruits became more pronounced as the Ni concentration increased in the solution so that the lower nitrate concentration was observed with urea-fed plant at 1 mg L^{-1} Ni concentration. Both N concentration and NR activity of young leaves were higher in urea-fed plants at 0.5 mg L^{-1} Ni concentration (Table 4). The lowest NR activity was observed in urea-fed plants without supplying Ni. Nitrate concentration of the leaves was not significantly affected by the treatments.

Although leaf chlorophyll index were low in both without Ni supplements and at 1 mg L^{-1} Ni concentration, the concentration of Ni in nitrate or urea-fed plant had no significant effect on chlorophyll index (Fig. 1).

Table 2. Effect of N source and Ni concentration on the vegetative characteristics and yield of cucumber plants

Treatments		Leaf FW (g plant^{-1})		Leaf DW (g plant^{-1})		Leaf area (cm^2)		Yield (kg plant^{-1})	
		RS189	Vikima	RS189	Vikima	RS189	Vikima	RS189	Vikima
Ni_0	Nitrate	331.0	330.1	23.0	20.8	6566.2b	6416.8b	1.9b	1.4b
$Ni_{0.5}$		359.6	330.7	24.1	22.4	6817.1b	6408.7b	1.9b	1.6a
Ni_1		353.3	321.8	23.1	22.2	6746.0b	6476.3b	1.6b	1.4b
Ni_0	Urea	374.3	332.7	26.4	23.4	7000.9b	6395.8b	1.9b	1.5b
$Ni_{0.5}$		365.0	327.3	26.8	22.5	7781.6a	6668.0a	2.1a	1.8a
Ni_1		363.6	315.0	24.9	21.7	7327.5b	6253.1b	1.4b	1.5b
F value									
N source		3.16ns	0.06 ns	2.6 *	0.68 ns	4.5*	0.31 ns	0.04 ns	0.46 ns
Ni		0.26 ns	0.64 ns	0.47 ns	0.12 ns	9.9**	8.30*	2.23**	2.80*
Ni×N		1.16 ns	0.08 ns	0.15 ns	1.20 ns	0.33 ns	1.34 ns	0.06 ns	0.43 ns

*, ** significant at P=0.05, P=0.01, respectively; ns- non significant

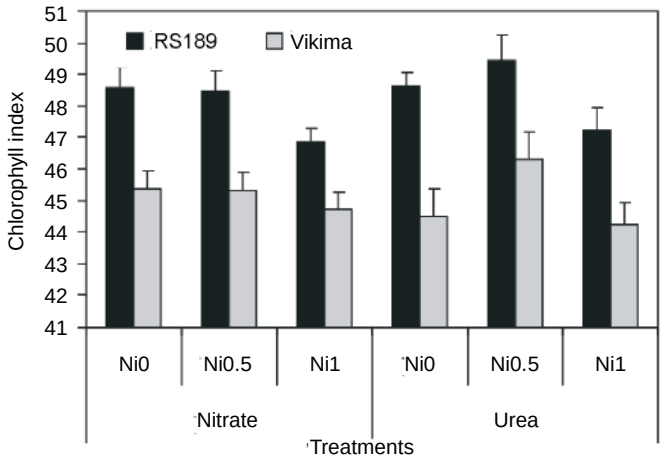

Fig. 1. Influence of N source and Ni concentraion on the chlorophyll index

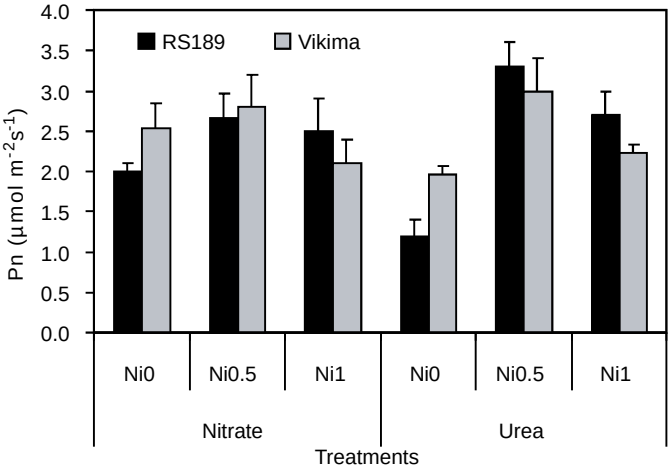

Fig. 2. Influence of N source and Ni concentraion on the Pn

The rate of Pn increased with the increase of the Ni concentration in the solution in urea-fed plants (Fig. 2). The rate of Pn impaired in the both without and high concentration of Ni in urea-fed plants. No significant difference in Pn was found between N sources. Both high and low concentration of Ni reduced the rate of Pn in the urea fed plants.

Discussion

Many studies on Ni have been focused on N metabolism and its related enzymes in higher plants in both monocots and dicots. Only very limited papers have been published concerning Ni application in relation to the yield and fruit quality. The results of this experiment indicated that Ni supplements enhanced the growth and yield of urea-fed plants by the increase of Pn and N concentration. Both high and low level of Ni impaired the growth of cucumber plants in terms of leaf area, fresh weight of leaves and yield. Therefore, supplying of Ni (at least 0.5 mg L^{-1}) to the nutrient solution for the urea-fed cucumber plant grown in hydroponics is required. In this work under Ni_0, the presence of Ni was observed in the leaves of plants implies that the plants were not strictly Ni limited. The presence of Ni in the fruits indicated that Ni is able to accumulate in the fruits however; the concentration of Ni in the fruits reduces as a result of rapid growth (dilution effect). The possible Ni contamination may be from chemical compounds (fertilizers) used for making solutions. Ni is one of the toxic compounds for human and environment hence, further attention should be paid in order to reduce the accumulation of Ni in the edible parts of the plant.

The quality and quantity of cucumber plants were improved with the supplements of Ni to the solution. The stimulating effect of a moderate Ni supply for urea-fed plant is well demonstrated (Shimada and Matsuo, 1985; Krogmeier *et al.*, 1991 and Xue *et al.*, 2000). The growth of cucumber plants without supplying Ni was not restored to the same level as for the nitrate-fed plant which agrees with earlier finding of Gerendas and Sattelmacher, (1977). Although Ni has been considered as an ultra-trace nutrient (Brown *et al.*, 1987), it is required at least 0.5 mg kg^{-1} DW for the normal growth of cucumber plant where urea is used as N source in the nutrient solution. This finding is in contrast with the report of Gerendas and Sattelmacher (1999) who observed non significant reduction in growth without Ni supply in *Brassica napus*. It seems that there may be differences among various plants in response to Ni supplements. Although Ni concentration in the leaves of the cucumber plants was higher where plant supplied with 1 mg L^{-1} Ni, no visible symptoms of toxicity was observed. It implies that cucumber plants are able to tolerate high concentration of Ni. Yang *et al.* (1997) found that the tolerance of some species to Ni concentration depends on the interaction of Ni with organic acids. The concentration of Ni in the cowpeas plants up to 3000 mg kg^{-1} DW is reported by Walker *et al.* (1985). Cucumber needs more N for the production of optimum yield, therefore any reduction in N content may reduce yield or plant

Table 3. Influence of N source and Ni concentraion on the fruit quality

Treatments		Fruit color Index		TSS (%)		Dry matter (%)		NO_3 (mg kg^{-1} DW)	
		RS189	Vikima	RS189	Vikima	RS189	Vikima	RS189	Vikima
Ni_0	Nitrate	41.5	35.7	5.0	5.5	5.2	5.3	69.7a	92.3a
$Ni_{0.5}$		44.1	31.8	5.1	5.1	5.3	5.2	43.6a	97.7a
Ni_1		43.7	33.7	5.3	5.0	5.6	5.1	40.0a	90.0a
Ni_0	Urea	44.8	33.0	4.8	4.3	5.6	5.2	38.0a	62.6a
$Ni_{0.5}$		44.3	38.0	5.0	5.0	5.3	5.1	32.0b	55.0b
Ni_1		45.8	32.5	4.6	5.0	5.6	5.0	30.6b	31.0b
F values									
N source		1.00 ns	0.56 ns	2.20*	8.5*	0.65 ns	0.22 ns	5.6*	6.71*
Ni		0.24 ns	1.10 ns	0.03 ns	0.2 ns	0.80 ns	1.04 ns	7.11**	3.28*
Ni×N		0.24 ns	1.70 ns	0.42 ns	1.7 ns	0.42 ns	0.03 ns	2.00 ns	0.85 ns

*, ** significant at *P*=0.05, *P*=0.01, respectively; ns- non significant

Table 4. Influence of N source and Ni concentraion on the concentration of N, NO_3 and NR activity in the leaves

Treatments		N (mg g^{-1}DW)		NO_3 (mg kg^{-1} DW)		NR (µmol h^{-1} g^{-1} FW)	
		RS189	Vikima	RS189	Vikima	RS189	Vikima
Ni_0	Nitrate	40.0 a	30.6 b	193.9	346.8	1.0 a	0.7 b
$Ni_{0.5}$		30.8 b	30.8 b	433.3	492.8	0.9 b	0.6 b
Ni_1		40.2 a	30.5 b	541.4	532.0	0.9 b	0.6 b
Ni_0	Urea	30.0 b	30.1 b	499.7	673.5	0.7 b	0.7b
$Ni_{0.5}$		40.4 a	40.6 a	221.7	320.0	1.2 a	1.0 a
Ni_1		30.6 b	30.0 b	280.0	492.8	0.8 b	0.6 b
F values							
N source		0.30 ns	2.70 ns	0.57 ns	1.80 ns	0.37 ns	0.02 ns
Ni		7.70*	6.90*	1.56 ns	1.35 ns	1.01*	1.40**
Ni×N		1.80 ns	0.82	1.50 ns	0.82 ns	0.09 ns	0.99 ns

*, ** significant at *P*=0.05, *P*=0.01, respectively; ns- non significant

growth. Xue *et al.* (2000) found that Ni supplements at 0.01 mg L^{-1} to the urea-fed tomato plants improved growth and N metabolism. The plants with urea without Ni supplements were smaller in size as reported by Gerendas and Sattelmacher, 1997, 1999; Mordy and Atta, 1999). There is no clear information about the critical toxicity concentration of Ni for cucumber; however the concentration of 1.2 mg kg^{-1} DW, with respect to cultivars, is most likely to reduce cucumber plant growth and yield. The reduction in growth of urea-fed plants without supplying Ni was reported by many researchers. They have demonstrated that urease activity as bio-indicator which is impaired in the plants grown with urea, consequently urea accumulate in large amounts in all parts of plants. The urea-fed plants without Ni supplements have substantially low amino acid N content in all parts of plants (Gerendas and Sattelmacher, 1997, 1999). These plants were not able to use the urea N provided due to reduced urease activity. In this work urease activity was not measured.

Dry matter percentage and colour of fruits were not affected by the treatments, however reduction in TSS was observed in the plants supplied with urea without Ni supplements. The reduced TSS in this treatment may be due to the reduced Pn which led to the reduction in sugar accumulation. The significant reduction of nitrate in the fruit of urea-fed plants, particularly with the increase of Ni concentration, is an important crucial issue. The reason of the reduction in nitrate concentration of fruit may be due to the increased activation of NR converting nitrate to amino acids (Marschner, 1995). A supplement of Ni to the urea-fed plants significantly reduced the nitrate concentration in spinach (Khan and Watanabe, 1999). In nitrate-fed plants the activity of NR was reduced when Ni concentration increased suggesting that the critical concentration of Ni in nitrate fed plant is much less than that of urea-fed plants. The reduction in nitrate in nitrate-fed plants by Ni supplements has not been reported. Changes in the content of organic acids and other solutes like nitrate might result from secondary events of disturbance in nitrogen metabolism in Ni-deficient plant (Marschner, 1995).

In this study, Ni concentration had no effect on chlorophyll index which is in contrast to Xue *et al.* (2000) findings in tomato. They reported that chlorophyll concentration increased in urea-fed plants because of urea assimilation. In this experiment, a presence of small amount of Ni in the leaves of without Ni supplemented plants could be the reason of the no reduced chlorophyll content. Furthermore, high concentration of N in all treatments may promote the chlorophyll content in the leaves.

The concentration of total N in urea-fed plants with Ni supplement at 0.5 mg L^{-1} was higher than those without Ni and high concentration of Ni, indicating that the absorption of urea is increased by Ni supplement up to 0.5 mg L^{-1} and then decreased at 1 mg L^{-1} Ni concentration. The activity of NR seems to play a key role in N metabolism in urea-fed plants (Table 3). The activity

of NR was strongly affected by Ni concentration in urea-fed plants suggesting the beneficial effect of Ni on the NR activity. Both high and low concentration of Ni in the plants reduced the activity of NR. NR is an enzyme that is regulated by several factors namely, enzyme synthesis, reversible inactivation, and concentration of substrate and effectors (Solomonson and Barber, 1990). Accumulation of urea without Ni supplements (Mordy and Atta-Aly, 1999; Watanabe and Shimada, 1990) and toxic effect of high concentration of Ni (1 mg L^{-1}) appeared to reduce Pn consequently reducing plant growth and yield.

Deprivation of Ni in barley led to lower content of amino acids and nitrate accumulation (Brown *et al.*, 1990). Changes in organic acids content and other solutes might result from secondary events of disturbance in nitrogen metabolism in Ni-deficient plant. It has not been known to what extent these various effects of Ni deficiency are directly related to the function of Ni in the urease. It has been demonstrated that urea is produced in a normal metabolite regardless supplements of N form. The ornithine cycle or degradation of protein for urea biosynthesis is likely to be general importance (Walker *et al.*, 1985). Therefore, Ni supplements might be playing an important role in all plant in the secondary events in nitrogen metabolism regardless N form nutrition. However, Gerendas and Sattelmacher (1999) suggested that Ni may not be strictly essential for *Brassica napus* plants receiving mineral N, or that the critical level is well below 25 µg kg^{-1} DW. The essentiality of Ni in nitrate assimilation in nitrate-fed plants seems to be unclear.

These results have important implications for the cucumber growers and physiologists. Use of Ni in the nutrient solutions containing urea has an important role to promote cucumber plants growth and increase the yield in commercials production. Furthermore, the reduction of nitrate concentration of the fruits in urea-fed plant with Ni supply at 0.5 mg L^{-1} improves the fruit quality. However, further attention should be paid to prevent the accumulation of Ni in the edible parts. Finally, impairing the NR enzyme in both high and low concentration of Ni and the effect of Ni on the nitrate assimilation need more investigations.

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