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Research Article

Effects of some micronutrients and macronutrients on the root-knot nematode, *Meloidogyne incognita*, in greenhouse cucumber (*Cucumis sativus* cv. Negin)

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Abstract: The effects of some micronutrients (iron, zinc and silicon) and macronutrients (nitrogen, phosphorus and potassium) were evaluated on the root-knot nematode, *Meloidogyne incognita* and plant growth parameters of cucumber (*Cucumis sativus* cv. Negin) in two independent trials. Each of iron, zinc and silicon micronutrients was used at 5 mg/kg of soil, as iron sequestrene (Fe-EDDHA), zinc sulfate ($ZnSO_4$) and sodium siliconate (Na_2O_3Si), respectively. Furthermore, nitrogen at 60, 120 and 180 mg/kg, phosphorus at 25, 50 and 75 mg/kg and potassium at 12.5, 25 and 37.5 mg/kg of soil were used as urea, triple superphosphate and potassium sulfate, respectively. At four-leaf stage seedlings, 8000 nematode eggs and juveniles (2 egg and juveniles/gr soil) were added around the roots. After 60-days, data analysis indicated silicon + iron, significantly reduced the number of galls/g of root by 55 and 42% compared to control, in the two experiments, respectively, but none of these treatments resulted in significant positive effects on the growth or yield of the studied cucumber cultivar. When macronutrients were evaluated, results showed that $N_{120}P_{25}K_{25}$ (120 mg/kg of Nitrogen, 25 mg/kg of phosphorus and 25 mg/kg of potassium) and $N_{120}P_{50}K_{25}$ (120 mg/kg of nitrogen, 50 mg/kg of phosphorus and 25 mg/kg of potassium) significantly reduced the number of galls by 96 and 81% (experiment 3) and 79 and 70% (experiment 4) when compared with control, respectively. These both treatments also improved cucumber growth parameters such as shoot dry and fresh weights, root fresh weight and fruit yield.

Keywords: Control, *Meloidogyne incognita*, micronutrient, macronutrient, greenhouse cucumber

Introduction

Root-knot nematodes, *Meloidogyne* spp., are the most important plant-parasitic nematodes attacking thousands of plant species (Back *et al.*, 2002; Hunt and Handoo, 2009). Four species namely *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, are of particular economic importance to vegetable production, but *M. incognita* is more widespread (Sikora and Fernandez, 2005).

Application of fertilizers or organic matter is an accepted practice for improving crop production and reducing nematode damage (Santana-Gomez *et al.*, 2013). Applications of certain fertilizers may be toxic to nematodes or suppress their multiplication and damage

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done to plants through changes in host nutrition (Marschner, 1997; Viaene *et al.*, 2013). Many researches have confirmed the efficacy of fertilizers, singly or in combination with other approaches, in the management of root-knot nematodes with resultant growth and yield improvement (Rodriguez-Kábana *et al.*, 1981; Rodriguez-Kábana, 1986; Asano and Moura, 1995; Crow *et al.*, 1996; Siddiqui *et al.*, 2001; Noweer and Hasabo, 2005; Nasr Esfahani and Ahmadi, 2005; Parveen *et al.*, 2008; Charegani *et al.*, 2010; Irshad *et al.*, 2011; Youssef *et al.*, 2012; Ismail *et al.*, 2011; Abolusoro *et al.*, 2013; Akhtar *et al.*, 2013).

This study was carried out to investigate the effects of the micronutrients (iron, zinc, silicon) and macronutrients (nitrogen, potassium and phosphorus) on *M. incognita* activity and infected cucumber cv. Negin at greenhouse condition.

Materials and Methods

Rearing and preparation of nematode population

Root-knot nematode culture was established from a single egg mass of *Meloidogyne incognita* on tomato plant (cv. Early Urbana). The nematodes were inoculated into plastic pots containing transplanted tomato seedlings (3- or 4-true leaf stage) by making three 2.5 cm deep holes around the seedlings. After 60 days, the aboveground portions of the plants were cut away and soil was gently washed from the roots with running tap water. Roots were cut into 2-3 cm pieces, placed into the flask, added some 0.5% NaOCl and shaken vigorously for 2 minutes (Hartman and Sasser, 1985; Hussey and Barker, 1973). After shaking, the nematode suspension was poured onto a sieve (with 850 µm apertures) nested on another sieve (with 25 µm apertures) and washed with tap water to eliminate residues of NaOCl. The eggs and second stage juveniles were collected in a beaker and their numbers estimated before using them for inoculation.

Cucumber growing condition and experimental setup

In total, four experiments (mentioned as experiments 1 to 4 in the text) were conducted in a greenhouse maintained at 25 ± 5 ºC. Plastic pots were filled with a mix of steam-sterilized field soil (loamy-sand with 66.5% sand, 17.1% silt, 16.4% clay, pH = 7.7, Ec = 0.93 S/m, with nutrient content of 0.04% N and also P, K, Z, Fe, Cu and Mn at 7, 290, 0.38, 4.02, 0.59 and 14.64 mg/kg, respectively) and river sand at 1:2 ratio. The macro and micro fertilizers were mixed into the soil mixture according to the required rate for each treatment. One cucumber seed (*Cucumber sativus* cv. Negin) was sown in each 20 cm diameter plastic pots filled with 4 kg of soil mixture in the experiments 1 to 3 and 25 cm diameter plastic pots each filled with 12 kg of soil mixture in the experiment 4 (to allow better extension of the root system of plants). The pots were laid out according to a factorial completely randomized design on benches in the greenhouse. Each pot was placed on a saucer and plants were watered once per day as required. Each treatment was replicated six, five, five and four times in the experiments 1 to 4, respectively.

Effect of micronutrients

In two sets of experiments; each of iron, zinc and silicon micronutrients was used at 5 mg/kg of soil, as iron sequestrene (Fe-EDDHA), zinc sulfate ($ZnSO_4$) and sodium siliconate (Na_2O_3Si), respectively. In the experiment 1, seven treatments were used *viz.* silicon, silicon + zinc, silicon + iron, zinc, zinc + iron, iron and control (no fertilization). In the experiment 2, another treatment was added as silicon + zinc + iron. At four-leaf stage seedlings of cucumber, 8000 nematode eggs and juveniles (2 eggs and juveniles/gr soil) were added around the roots by making three 2.5 cm deep holes around the seedlings. This nematode inoculum is greater than 0.5-1 egg and juveniles/gr of soil which has been mentioned for economic threshold level of root-knot nematodes in literature (Sikora and Fernández, 2005), to compensate for the eggs and juveniles that might be inactivated or lost during inoculation procedure.

Effect of macronutrients

Nitrogen (N), phosphorus (P) and potassium (K) were used as urea, triple superphosphate and potassium sulfate, respectively. In the experiments 3 and 4, seventeen treatments were assigned *viz.* N_{60} (60 mg/kg of N), N_{120} (120 mg/kg of N), N_{180} (180 mg/kg of N), P_{25} (25 mg/kg of P), P_{50} (50 mg/kg of P), P_{75} (75 mg/kg of P), $K_{12.5}$ (12.5 mg/kg of K), K_{25} (25 mg/kg of K), $K_{37.5}$ (37.5 mg/kg of K), $N_{120}P_{50}K_{12.5}$, $N_{120}P_{50}K_{25}$, $N_{120}P_{50}K_{37.5}$, $N_{120}P_{25}K_{25}$, $N_{120}P_{75}K_{25}$, $N_{60}P_{50}K_{25}$, $N_{180}P_{50}K_{25}$ and control (no fertilization). The time and mode of the nematode inoculation was same as described for themicro nutrients experiments.

Data collection

The assessment of plant data and nematode indices was done 60 days after inoculation. Plants were harvested, transferred into paper envelopes and plant growth factors (shoot fresh and dry weights, root fresh weight, and fruit weight) were recorded. For determination of shoot dry weight, envelopes were maintained in an oven at 72 ºC for 48 hours before weighing. For fruit weight index, the plants were monitored daily and each fruit approximately 10 cm in length, was harvested and weighed. The number of nematodes per 100 g of soil was determined according to the method developed by Whitehead and Hemming (1965). The number of eggs per g of root was also determined by cutting the roots into pieces 2-3 cm long of which 1 g was randomly selected. The eggs were then extracted by agitating the root pieces in a 0.5% NaOCl solution for 2 min (Hussey and Barker, 1973), sieved as described earlier and counted. The reproduction factor of the nematode and gall index were calculated according to the methods described by Sasser and Taylor (1978) and Hussey and Janssen (2002).

Statistical analysis

Data on plant growth and nematode indices of the experiments were analyzed in completely randomized design and subjected to separate analysis of variance (ANOVA). Means were compared with Duncan□s multiple range tests using 9.1 version of the SAS program.

Results

Effect of micronutrients

In the experiment 1, shoot fresh weight was increased to the highest level in iron + zinc and iron treatments (inoculated with nematode), and these treatments showed significant differences with all other treatments, except with those in silicon + zinc (non-inoculated) treatment. Other plant growth variables did not show any significant difference in any of the treatments (Table 1). The measured plant growth variables showed no significant differences in the experiment 2 (Table 2).

Regarding nematode indices, in the experiment 1, the lowest number of eggs/g of root was observed in zinc treatment. The zinc, silicon and silicon + iron treatments resulted significant reduction in the number of galls/g of root, when compared to the other treatments. A significant reduction was also observed for the number of egg masses/g of root in silicon + iron treatment. Similar trend was evident for the number of juveniles in soil in the silicon + zinc treatment. The lowest reproduction factor was 36 for zinc treatment, but any significant difference was not observed among the treatments (Table 3). In the experiment 2, no significant differences were observed among treatments when comparing the means of the different nematode indices. The effective role of zinc in reducing the number of the nematode eggs, galls and reproduction factor was not observed in the experiment 2, unlike for experiment 1 (Table 4). Infestation of plants with spider mite (*Tetranychus urticae*) may have caused an adverse effect on plant growth, during the experiment 2.

On the whole, results indicated that although some micronutrient treatments, especially silicon + iron, significantly reduced the number of galls per gr of root by 55 and 42% in comparison with control, in the experiments 1 and 2, respectively, none of micronutrients resulted in significant positive effects on the growth or yield of the studied cucumber cultivar.

Table 1 The effects of silicon, zinc and iron applications (5 mg/kg of soil) on growth parameters of cucumber plants (cv. Negin), inoculated (+) or non-inoculated (-) with *Meloidogyne incognita*, under greenhouse conditions (experiment 1).

Treatment	Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)		Fruit yield (g)	
	+	-	+	-	+	-	+	-
Silicon	100.5 ± 15.5 ab	94.9 ± 15.0 ab	20.8 ± 23.3 bc	16.6 ± 18.5 c	38.4 ± 17.9 ab	39.1 ± 25.4 ab	120.6 ± 17.5 ab	139.2 ± 26.2 a
Silicon + zinc	97.4 ± 10.1 ab	85.3 ± 17.2 b	14.2 ± 6.3 c	17.6 ± 21.6 c	40.1 ± 15.7 ab	36.2 ± 18.8 b	94.2 ± 32.2 ab	129.2 ± 19.2 a
Silicon + iron	112.0 ± 14.0 ab	100.8 ± 9.6 ab	20.5 ± 24.0 bc	27.8 ± 11.8 a	41.9 ± 19.9 ab	43.5 ± 18.5 ab	104.6 ± 24.2 ab	122.8 ± 23.5 ab
Zinc	107.8 ± 12.4 ab	99.0 ± 13.9 ab	17.2 ± 12.5 c	25.5 ± 15.2 ab	47.8 ± 15.1 ab	41.5 ± 16.5 ab	127.0 ± 15.9 ab	121.4 ± 25.1 ab
Zinc + iron	120.6 ± 11.3 a	106.3 ± 10.1 ab	20.2 ± 8.8 bc	18.8 ± 14.9 bc	55.0 ± 25.3 a	44.1 ± 19.9 ab	91.8 ± 24.5 ab	120.8 ± 15.4 ab
Iron	115.8 ± 13.9 a	113.5 ± 17.2 a	21.0 ± 21.7 a-c	20.2 ± 25.4 bc	52.8 ± 19.0 ab	48.9 ± 15.5 ab	109.0 ± 22.2 ab	69.9 ± 45.3 b
Control	112.2 ± 6.1 ab	106.0 ± 10.8 ab	19.0 ± 5.9 bc	17.0 ± 9.5 c	42.3 ± 25.8 ab	41.5 ± 23.9 ab	113.4 ± 24.3 ab	137.2 ± 32.7 a

Data are the average ± CV of six replicates.
Values followed by the same letters in each column are not significantly different (Duncan□s Multiple Range Test, P ≤ 0.05).

Table 2 The effects of silicon, zinc and iron applications (5 mg/kg of soil) on growth parameters of cucumber plants (cv. Negin), inoculated (+) or non-inoculated (-) with *Meloidogyne incognita*, under greenhouse conditions (experiment 2).

Treatment	Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)		Fruit yield (g)	
	+	-	+	-	+	-	+	-
Silicon	82.1 ± 23.2 a	64.6 ± 10.9 ab	13.7 ± 16.4 a	10.6 ± 15.3 a-c	16.9 ± 49.1 a-c	18.4 ± 25.1 a-c	42.7 ± 27.7 b	61.9 ± 22.1 ab
Silicon + zinc	53.7 ± 32.9 ab	77.1 ± 4.3 ab	11.6 ± 16.1 a-c	10.5 ± 20.50 a-d	16.1 ± 41.5 a-c	15.9 ± 55.9 a-c	39.4 ± 33.0 b	64.4 ± 31.8 ab
Silicon + iron	48.9 ± 33.6 b	54.1 ± 27.9 ab	5.9 ± 32.8 d	7.7 ± 43.4 cd	8.7 ± 86.7 c	10.3 ± 79.7 bc	37.2 ± 24.9 b	65.1 ± 40.8 ab
Zinc	76.4 ± 5.4 ab	72.5 ± 17.0 ab	12.4 ± 10.0 a-c	12.5 ± 21.9 ab	24.1 ± 34.2 a-c	22.8 ± 33.7 a-c	74.1 ± 16.4 ab	63.3 ± 105.4 ab
Zinc + iron	66.2 ± 18.0 ab	64.8 ± 15.0 ab	12.1 ± 15.6 a-c	12.7 ± 10.8 ab	27.4 ± 34.9 ab	32.1 ± 22.5 a	56.9 ± 32.6 ab	106.5 ± 13.3 a
Iron	66.9 ± 12.5 ab	59.5 ± 30.5 ab	13.3 ± 19.8 ab	11.5 ± 13.0 a-c	21.6 ± 43.5 a-c	16.5 ± 37.1 a-c	63.5 ± 39.4 ab	54.1 ± 26.4 ab
Silicon + zinc + iron	55.8 ± 32.2 ab	66.0 ± 11.3 ab	8.6 ± 26.0 b-d	10.9 ± 9.9 a-c	19.4 ± 33.0 a-c	15.8 ± 59.1 a-c	52.1 ± 72.7 ab	53.6 ± 47.9 ab
Control	67.5 ± 14.1 ab	76.7 ± 18.7 ab	11.1 ± 11.2 a-c	12.6 ± 21.0 ab	18.4 ± 45.7 a-c	22.3 ± 50.1 a-c	30.7 ± 53.2 b	46.7 ± 53.0 b

- Data are the average ± CV of five replicates.
- Values followed by the same letters in each column are not significantly different (Duncan□s Multiple Range Test, P ≤ 0.05).

Table 3 The effects of silicon, zinc and iron applications (5 mg/kg of soil) on indices of *Meloidogyne incognita*, in the infected cucumber (cv. Negin), under greenhouse conditions (experiment 1).

Treatment	Eggs/g root	Galls/g root	Egg masses/g root	Juveniles/pot soil	Reproduction factor
Silicon	9400 ± 25.2 cd	81 ± 20.2 c	36 ± 41.6 cd	2880 ± 245.0 b	45 ± 49.0 a
Silicon + zinc	15167 ± 11.8 ab	150 ± 16.7 ab	59 ± 29.0 bc	960 ± 155.4 b	59 ± 53.7 a
Silicon + iron	11450 ± 28.9 bc	84 ± 21.6 c	33 ± 40.6 d	20021 ± 59.2 a	64 ± 25.4 a
Zinc	5633 ± 34.9 e	79 ± 27.2 c	39 ± 52.0 cd	12384 ± 82.8 ab	36 ± 58.9 a
Zinc + iron	7867 ± 11.6 c-e	165 ± 14.5 ab	66 ± 15.0 bc	9941 ± 60.1 ab	51 ± 39.5 a
Iron	6883 ± 17.5 de	133 ± 16.5 b	91 ± 18.5 b	4651 ± 82.8 b	53 ± 40.9 a
Control	17200 ± 11.9 a	186 ± 15.2 a	162 ± 17.9 a	8085 ± 99.5 ab	73 ± 36.3 a

- Data are the average ± CV of six replicates.
- Values followed by the same letters in each column are not significantly different (Duncan□s Multiple Range Test, P ≤ 0.05).

Table 4 The effects of silicon, zinc and iron applications (5 mg/kg of soil) on indices of *Meloidogyne incognita*, in the infected cucumber (cv. Negin), under greenhouse conditions. (experiment 2).

Treatment\Variable	Eggs/g root	Galls/g root	Egg masses/g root	Juveniles / pot soil	Reproduction factor
Silicon	35576 ± 61.9a	123 ± 69.8c	97 ± 64.2c	53634 ± 114.0a	76 ± 38.2a
Silicon + zinc	38117 ± 54.0a	218 ± 18.7a-c	182 ± 30.2bc	54138 ± 55.5a	72 ± 22.5a
Silicon + iron	40096 ± 60.0a	152 ± 23.1bc	141 ± 29.4bc	29568 ± 125.1a	47 ± 96.2a
Zinc	38729 ± 34.4a	312 ± 21.7a	296 ± 20.4a	31864 ± 51.9a	119 ± 41.0a
Zinc + iron	20283 ± 46.1a	150 ± 52.2bc	103 ± 25.9c	79338 ± 50.8a	79 ± 71.2a
Iron	28288 ± 44.3a	100 ± 18.2bc	88 ± 41.8c	32214 ± 76.7a	77 ± 49.1a
Silicon + zinc + iron	40076 ± 45.5a	176 ± 20.2bc	144 ± 13.0bc	30632 ± 72.0a	71 ± 41.2a
Control	37988 ± 71.9a	262 ± 23.2ab	236 ± 29.0ab	58646 ± 84.0a	74 ± 40.1a

- Data are the average ± CV of five replicates.
- Values followed by the same letters in each column are not significantly different (Duncan□s Multiple Range Test, P ≤ 0.05).

Effect of macronutrients

In experiment 3, the highest values of shoot fresh weight and shoot dry weight were obtained in the plants inoculated with *M. incognita* and treated with $N_{120}P_{50}K_{25}$ and $N_{120}P_{75}K_{25}$ fertilizers, respectively. The root fresh weight showed a significant increase also in $N_{120}P_{50}K_{25}$ treatment, but the highest yield was obtained in $N_{120}P_{50}K_{12.5}$ treatment (Table 5). In the experiment 4, $N_{120}P_{50}K_{37.5}$ treatment attained the highest shoot fresh weight, shoot dry weight and fruit yield in the plants inoculated with *M. incognita*, but the highest root fresh weight was obtained in the plants inoculated with *M. incognita* and treated with $N_{120}P_{25}K_{25}$ fertilizer (Table 6).

Analysis of the nematode indices revealed that, in the experiment 3, $N_{120}P_{25}K_{25}$ showed a significant reduction in the number of eggs, galls and egg masses per g of root, as well as in the nematode reproduction factor. The lowest number of juveniles in soil was observed in $N_{120}P_{50}K_{12.5}$ treatment, although any significant difference was not observed among treatments (Table 7). In the experiment 4, the number of eggs, galls and juveniles was significantly lower in $N_{120}P_{25}K_{25}$ treatment than those in the other treatments. However, $N_{120}P_{50}K_{25}$ was the most effective treatment for reduction of *M. incognita* reproduction factor (Table 8).

In summary, results showed that $N_{120}P_{25}K_{25}$ (120 mg/kg of Nitrogen, 25 mg/kg of phosphorus and 25 mg/kg of potassium) and $N_{120}P_{50}K_{25}$ (120 mg/kg of nitrogen, 50 mg/kg of phosphorus and 25 mg/kg of potassium) significantly reduced the number of galls by 96 and 81% (experiment 3), and 79 and 70% (experiment 4) when compared with control, respectively. These both treatments resulted in the improvement of cucumber parameters such as shoot dry and fresh weights, root fresh weight and fruit yield.

Discussion

In this study, some micronutrient treatments, typically silicon + iron, resulted in drastic reduction in the number of eggs, galls, egg masses and juveniles of the nematode, but they had no significant positive effect on the plant growth indices. On the other hand, some treatments of silicon + iron showed a significant reduction in the plant growth parameters when compared with control; this might have resulted from the adverse effects of the combined application of these two mineral elements with each other, as already has been shown for inhibitory effects of the high concentrations of magnesium on the availability of potassium, manganese and calcium to plants (Persson and Olsson, 2000). In the soil, nematodes are attracted to their hosts by the concentration gradient formed by root exudates, which provides a recognition or repellency signal for nematodes (Santana-Gomes *et al.*, 2013). Although it is not exactly clear if mineral nutrients are involved in this process, some evidences are available on the role of nematodes on root system development. For example, nematodes are cited as the main agents responsible for potassium deficiency in apples (Santana-Gomes *et al.*, 2013). In some plants, attack by nematode can cause significant losses, but does not affect plant growth in the presence of adequate levels of available macronutrients or micronutrients (Asano and Moura, 1995; Santana-Gomes *et al.*, 2013).

Table 5 The effects of nitrogen, phosphorus and potassium applications on growth parameters of cucumber plants (cv. Negin), inoculated (+) or non-inoculated (-) with *Meloidogyne incognita*, under greenhouse conditions (experiment 3).

Treatment (mg/kg of soil)	Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)		Fruit yield (g)	
	+	-	+	-	+	-	+	-
N_{60}	70.2 ± 29.2 g-j	64.1 ± 15.3 g-k	10.3 ± 23.4 g-n	10.0 ± 16.3 g-n	26.1 ± 34.0 a-e	23.8 ± 38.2 a-e	8.7 ± 223.6 e	25.0 ± 100.1 de
N_{120}	41.9 ± 17.9 kl	80.78 ± 11.9 d-h	5.8 ± 25.0 no	12.2 ± 6.3 e-k	11.3 ± 46.6 de	28.6 ± 23.9 a-e	0e	36.0 ± 113.1 c-e
N_{180}	89.1 ± 16.8 b-g	49.6 ± 16.4 j-l	12.4 ± 13.0 d-j	7.5 ± 16.6 l-o	28.4 ± 38.2 a-e	12.1 ± 65.2 de	13.2 ± 184.6 e	20.8 ± 168.6 e
P_{25}	50.7 ± 2.9 j-l	53.5 ± 17.0 i-l	8.6 ± 27.9 i-o	10.8 ± 19.4 f-m	22.8 ± 19.2 a-e	29.1 ± 33.9 a-e	1.9 ± 223.6 e	8.9 ± 223.6 e
P_{50}	54.2 ± 5.3 i-l	47.6 ± 9.3 j-l	11.6 ± 20.1 f-l	9.2 ± 16.9 h-o	20.4 ± 30.7 a-e	23.1 ± 24.4 a-e	0.0 ± 0 e	20.2 ± 105.0 e
P_{75}	62.1 ± 14.9 h-k	54.3 ± 9.5 i-l	11.4 ± 23.7 f-l	12.0 ± 11.4 f-l	24.6 ± 35.0 a-e	30.4 ± 21.0 a-d	4.2 ± 223.6 e	29.1 ± 94.6 de
$K_{12.5}$	39.1 ± 5.2 kl	36.9 ± 17.5 l	6.4 ± 11.4 m-o	6.3 ± 23.3 m-o	16.5 ± 25.9 b-e	23.3 ± 38.4 a-e	18.3 ± 223.6 e	4.7 ± 141.4 e
K_{25}	47.5 ± 11.6 j-l	43.8 ± 6.3 kl	7.5 ± 27.3 l-o	7.7 ± 9.8 j-o	21.4 ± 50.3 a-e	22.3 ± 9.9 a-e	0.0 ± 0 e	0.0 ± 0 e
$K_{37.5}$	50.0 ± 10.7 j-l	39.9 ± 21.2 kl	7.5 ± 10.8 k-o	6.5 ± 20.0 m-o	20.7 ± 34.9 a-e	15.2 ± 39.4 c-e	5.3 ± 223.6 e	9.5 ± 182.6 e
N_{120} P_{50} $K_{12.5}$	104.6 ± 6.3 a-e	99.9 ± 10.0 a-f	17.7 ± 5.5 ab	16.8 ± 12.1 a-e	38.7 ± 17.8 ab	33.0 ± 39.5 a-d	178.1 ± 10.6 ab	157.1 ± 4.5 a-c
N_{120} P_{50} K_{25}	108.7 ± 10.7 a-c	112.6 ± 10.3 a-e	16.9 ± 16.5 a-d	17.8 ± 11.1 ab	39.4 ± 40.6 a	30.7 ± 42.0 a-d	157.4 ± 22.4 a-c	231.3 ± 45.5 a
N_{120} P_{50} $K_{37.5}$	102.1 ± 8.6 a-f	80.2 ± 13.7 e-h	17.2 ± 12.4 a-c	12.9 ± 11.8 c-i	34.6 ± 11.3 a-c	24.7 ± 53.3 a-e	148.7 ± 54.8 a-d	169.7 ± 44.0 ab
N_{120} P_{25} K_{25}	105.8 ± 12.6 a-d	87.7 ± 13.3 b-g	17.8 ± 10.6 ab	13.6 ± 15.9 b-h	22.7 ± 34.3 a-e	27.1 ± 46.1 a-e	161.0 ± 41.8 a-c	119.2 ± 28.0 a-e
N_{120} P_{75} K_{25}	102.0 ± 8.6 a-f	116.9 ± 20.4 a	17.7 ± 7.1 ab	19.3 ± 18.8 a	30.5 ± 29.7 a-d	28.8 ± 27.4 a-e	112.9 ± 108.5 a-e	190.0 ± 73.7 ab
N_{60} P_{50} K_{25}	77.3 ± 12.0 f-i	84.4 ± 17.3 c-h	13.9 ± 9.1 b-g	15.0 ± 10.9 a-f	25.1 ± 59.3 a-e	37.5 ± 34.3 a-c	91.0 ± 82.5 b-e	71.3 ± 91.3 b-e
N_{180} P_{50} K_{25}	85.8 ± 16.5 a-c	108.8 ± 3.9 a-c	14.6 ± 17.4 a-g	16.9 ± 17.4 a-d	29.5 ± 36.6 a-e	36.9 ± 11.7 a-c	31.1 ± 69.0 de	224.4 ± 31.1 a
Control	40.2 ± 13.0 kl	31.1 ± 28.1 l	6.64 ± 24.4 m-o	4.8 ± 34.0 o	17.6 ± 22.2 a-e	7.6 ± 77.8 e	0.00 ± 0 e	2.78 ± 223.6 e

- Data are the average ± CV of five replicates.
- Values followed by the same letters in each column are not significantly different (Duncan□s Multiple Range Test, P ≤ 0.05).

Table 6 The effects of nitrogen, phosphorus and potassium applications on growth parameters of cucumber plants (cv. Negin), inoculated (+) or non-inoculated (-) with *Meloidogyne incognita*, under greenhouse conditions (experiment 4).

Treatment (mg/kg of soil)	Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)		Fruit yield (g)	
	+	-	+	-	+	-	+	-
N_{60}	104.6 ± 5.0 h-l	102.0 ± 5.9 i-m	17.6 ± 2.1 d-h	16.0 ± 5.1 d-j	22.3 ± 14.3 f-i	20.6 ± 11.9 h-k	130.6 ± 15.0 g-k	92.4 ± 39.5 g-l
N_{120}	105.0 ± 6.8 g-k	96.7 ± 12.9 i-m	17.5 ± 6.2 d-h	20.0 ± 11.0 c-g	16.4 ± 4.7 i-l	14.5 ± 6.8 i-m	90.1 ± 13.3 g-l	91.0 ± 31.8 g-l
N_{180}	101.7 ± 7.0 i-m	108.6 ± 6.5 f-j	20.1 ± 5.0 c-g	20.1 ± 6.5 c-f	22.0 ± 9.6 f-i	16.0 ± 6.8 i-m	92.6 ± 45.4 g-l	164.2 ± 27.2 f-i
P_{25}	101.2 ± 7.1 i-m	82.4 ± 6.9 mn	20.8 ± 6.5 c-e	14.1 ± 7.6 g-j	15.7 ± 7.5 i-m	13.0 ± 7.9 k-m	10.0 ± 200.0 l	14.0 ± 55.2 l
P_{50}	98.4 ± 9.8 i-m	116.3 ± 6.6 d-i	18.0 ± 5.0 d-h	20.4 ± 5.4 c-f	20.5 ± 8.4 h-k	21.6 ± 3.3 f-j	62.5 ± 33.8 i-l	63.7 ± 24.4 h-l
P_{75}	108.4 ± 6.3 f-j	111.7 ± 8.6 e-j	20.6 ± 2.8 c-f	21.4 ± 1.8 cd	20.5 ± 10.0 h-k	14.1 ± 9.7 j-m	44.1 ± 24.1 j-l	73.0 ± 27.7 h-l
$K_{12.5}$	97.0 ± 6.3 i-m	85.9 ± 4.6 k-n	14.6 ± 9.4 f-j	13.8 ± 13.3 h-j	19.1 ± 7.6 h-l	14.7 ± 5.6 i-m	22.7 ± 35.7 kl	54.1 ± 26.9 i-l
K_{25}	84.8 ± 6.1 l-n	99.7 ± 3.2 i-m	14.0 ± 5.0 h-j	15.1 ± 6.8 e-j	20.4 ± 10.8 h-k	14.5 ± 5.8 i-m	25.4 ± 19.0 kl	20.4 ± 24.8 kl
$K_{37.5}$	61.1 ± 5.8 o	72.5 ± 9.2 no	10.2 ± 13.0 j	11.5 ± 13.7 h-j	11.7 ± 14.3 lm	8.6 ± 18.1 m	21.0 ± 82.6 kl	33.2 ± 68.9 j-l
N_{120} P_{50} $K_{12.5}$	131.8 ± 5.9 a-e	124.8 ± 3.2 c-g	31.0 ± 5.0 ab	33.3 ± 6.3 a	29.0 ± 7.0 d-f	20.6 ± 14.7 h-k	202.5 ± 17.9 e-g	320.0 ± 25.8 a-d
N_{120} P_{50} K_{25}	126.2 ± 4.4 b-f	151.4 ± 6.4 a	32.4 ± 3.7 a	35.4 ± 5.7 a	25.5 ± 6.6 e-h	33.3 ± 10.2 de	326.1 ± 21.4 a-d	428.8 ± 14.8 a
N_{120} P_{50} $K_{37.5}$	146.3 ± 2.1 ab	141.1 ± 5.5 a-c	36.0 ± 5.4 a	35.6 ± 9.2 a	53.0 ± 4.6 b	30.3 ± 10.1 de	414.8 ± 17.3 ab	334.6 ± 9.1 a-d
N_{120} P_{25} K_{25}	134.0 ± 4.5 a-d	127.4 ± 7.1 b-f	35.9 ± 6.0 a	34.1 ± 10.0 a	61.6 ± 6.7 a	42.8 ± 6.2 c	259.9 ± 8.0 d-f	330.9 ± 21.4 a-d
N_{120} P_{75} K_{25}	135.9 ± 6.9 a-d	136.7 ± 6.7 a-c	33.4 ± 14.8 a	35.8 ± 6.3 a	33.7 ± 7.3 d	28.7 ± 9.8 d-g	378.1 ± 23.7 a-c	305.9 ± 18.0 b-e
N_{60} P_{50} K_{25}	97.8 ± 6.9 i-m	123.8 ± 7.0 c-h	21.5 ± 13.2 cd	25.8 ± 7.1 bc	22.2 ± 19.8 f-i	21.1 ± 20.3 g-j	118.6 ± 23.0 g-l	144.3 ± 19.9 g-j
N_{180} P_{50} K_{25}	137.0 ± 5.6 a-c	123.2 ± 5.0 c-h	35.0 ± 13.4 a	34.2 ± 13.6 a	34.9 ± 14.4 d	21.7 ± 37.9 f-j	300.7 ± 26.8 c-e	177.4 ± 11.0 f-h
Control	73.7 ± 13.0 no	92.4 ± 6.1 j-n	13.2 ± 8.9 h-j	17.4 ± 10.4 d-i	14.1 ± 22.7 j-m	13.2 ± 6.7 k-m	5.3 ± 200.0 l	36.9 ± 23.7 j-l

- Data are the average ± CV of four replicates.
- Values followed by the same letters in each column are not significantly different (Duncan□s Multiple Range Test, P ≤ 0.05).

Table 7 The effects of nitrogen, phosphorus and potassium applications on indices of *Meloidogyne incognita*, in the infected cucumber (cv. Negin), under greenhouse conditions (experiment 3).

Treatment (mg/kg of soil)	Eggs / g root	Galls / g root	Egg masses / g root	Juveniles / pot soil	Reproduction factor
N_{60}	55462 ± 28.7 b-d	178 ± 32.1 b-d	142 ± 40.7 bc	1640 ± 103.8 a	190 ± 54.9 a
N_{120}	105912 ± 41.9 a	247 ± 47.9 ab	220 ± 45.1 ab	2953 ± 162.3 a	132 ± 38.9 ab
N_{180}	36823 ± 35.5 b-e	138 ± 8.2 b-d	106 ± 19.9 cd	703 ± 96.1 a	123 ± 32.6 ab
P_{25}	53921 ± 31.9 b-d	115 ± 50.9 de	113 ± 53.1 b-d	5556 ± 121.9 a	150 ± 25.0 a
P_{50}	61724 ± 14.9 a-c	129 ± 63.9 ce	120 ± 66.2 b-d	4507 ± 168.2 a	160 ± 36.6 a
P_{75}	38663 ± 29.4 b-e	125 ± 18.6 ce	121 ± 16.7 bc	1285 ± 161.5 a	113 ± 30.5 ab
$K_{12.5}$	51430 ± 34.9 b-d	96 ± 29.0 de	92 ± 31.4 cd	3937 ± 137.0 a	100 ± 19.1 ab
K_{25}	70292 ± 51.8 ab	234 ± 19.5 bc	222 ± 18.5 ab	4905 ± 134.9 a	182 ± 79.5 a
$K_{37.5}$	43647 ± 28.1 b-e	124 ± 26.0 c-e	105 ± 25.6 cd	3584 ± 139.2 a	117 ± 46.0 ab
N_{120} P_{50} $K_{12.5}$	31140 ± 47.5 b-e	96 ± 44.4 de	82 ± 51.0 cd	268 ± 91.7 a	150 ± 51.9 a
N_{120} P_{50} K_{25}	10003 ± 63.3 b-e	67 ± 10.9 de	47 ± 36.8 cd	883 ± 192.1 a	55 ± 70.9 ab
N_{120} P_{50} $K_{37.5}$	33902 ± 30.8 b-e	161 ± 31.1 bd	136 ± 35.9 bc	408 ± 159.9 a	146 ± 27.9 a
N_{120} P_{25} K_{25}	3380 ± 132.9 e	14 ± 26.7 e	10 ± 40.1 d	1498 ± 136.9 a	7 ± 105.1 b
N_{120} P_{75} K_{25}	18388 ± 52.6 c-e	78 ± 50.6 de	61 ± 56.2 cd	673 ± 52.2 a	64 ± 34.6 ab
N_{60} P_{50} K_{25}	27449 ± 73.0 b-e	108 ± 21.8 de	92 ± 35.9 cd	197 ± 223.6 a	69 ± 45.7 ab
N_{180} P_{50} K_{25}	21676 ± 24.2 c-e	102 ± 22.2 de	70 ± 19.9 cd	736 ± 91.0 a	76 ± 20.0 ab
Control	69966 ± 50.2 ab	353 ± 22.5 a	328 ± 24.8 a	4694 ± 44.8 a	152 ± 45.2 a

- Data are the average ± CV of five replicates.
- Values followed by the same letters in each column are not significantly different (Duncan□s Multiple Range Test, $P \leq 0.05$).

Table 8 The effects of nitrogen, phosphorus and potassium applications on indices of *Meloidogyne incognita*, in the infected cucumber (cv. Negin), under greenhouse conditions (experiment 4).

Treatment (mg/kg of soil)	Eggs / g root	Galls / g root	Egg masses / g root	Juveniles / pot soil	Reproduction factor
N_{60}	33903 ± 34.7 a-e	217 ± 38.8 b-d	188 ± 30.9 b-d	48660 ± 50.7 b-d	48 ± 35.9 a-d
N_{120}	45082 ± 19.6 ab	157 ± 11.0 c-f	145 ± 13.1 c-f	113220 ± 17.3 ab	54 ± 15.2 a-c
N_{180}	28797 ± 69.6 b-e	202 ± 13.4 c-e	178 ± 13.7 c-e	40200 ± 39.4 b-d	39 ± 57.99 b-d
P_{25}	28641 ± 23.6 b-e	127 ± 14.7 d-f	91 ± 12.4 c-f	45780 ± 42.4 b-d	30 ± 16.5 cd
P_{50}	40678 ± 16.6 a-d	244 ± 28.6 bc	216 ± 30.1 b-c	94560 ± 49.3 a-c	57 ± 25.8 a-c
P_{75}	26379 ± 13.8 b-e	161 ± 2.6 c-f	139 ± 14.8 c-f	64500 ± 43.6 b-d	37 ± 7.2 b-d
$K_{12.5}$	42355 ± 28.7 a-c	161 ± 15.5 c-f	126 ± 22.5 c-f	95280 ± 42.2 a-c	55 ± 17.9 a-c
K_{25}	45504 ± 33.2 ab	169 ± 18.0 c-f	138 ± 35.0 c-f	155970 ± 28.0 a	69 ± 19.7 a
$K_{37.5}$	40948 ± 42.2 a-d	309 ± 7.3 b	275 ± 7.6 b	98520 ± 37.5 a-c	38 ± 20.5 b-d
N_{120} P_{50} $K_{12.5}$	16684 ± 17.9 c-e	135 ± 33.4 d-f	131 ± 36.2 c-f	37800 ± 100.5 cd	31 ± 10.4 cd
N_{120} P_{50} K_{25}	14392 ± 48.5 e	156 ± 15.9 c-f	123 ± 27.3 d-f	28620 ± 40.5 cd	24 ± 44.6 d
N_{120} P_{50} $K_{37.5}$	17298 ± 23.3 c-e	98 ± 24.3 f	79 ± 20.8 f	17940 ± 6.1 d	52 ± 26.1 a-d
N_{120} P_{25} K_{25}	10453 ± 16.2 e	93 ± 25.7 f	79 ± 32.1 f	13680 ± 131.6 d	38 ± 15.4 b-d
N_{120} P_{75} K_{25}	15259 ± 38.4 de	113 ± 11.7 ef	97 ± 12.3 ef	31980 ± 55.5 cd	32 ± 31.6 b-d
N_{60} P_{50} K_{25}	24209 ± 50.2 b-e	165 ± 31.8 c-f	149 ± 28.0 c-f	39360 ± 70.7 cd	33 ± 35.7 b-d
N_{180} P_{50} K_{25}	16173 ± 24.3 de	147 ± 21.3 c-f	121 ± 27.7 d-f	39360 ± 67.5 cd	36 ± 24.1 b-d
Control	54609 ± 9.1 a	440 ± 8.8 a	433 ± 8.0 a	84900 ± 28.3 a-d	61 ± 10.7 ab

- Data are the average ± CV of four replicates.
- Values followed by the same letters in each column are not significantly different (Duncan□s Multiple Range Test, $P \leq 0.05$).

Among all treatments, $N_{120}P_{25}K_{25}$ was the most effective treatment for reduction of the nematode indices, but the least reproduction factor was observed in another treatment, $N_{120}P_{50}K_{25}$. This unexpected result can be explained as the increased weight of fresh root in $N_{120}P_{25}K_{25}$ resulted in a well-developed root system, which provides more substrate for the formation of more galls, subsequently more eggs and finally the highest value of the nematode reproduction factor. Furthermore, plants treated with the combined fertilizers showed significant increases in root fresh weight, compared with single application of these nutrients; this increased root weight can be also attributed to the formation of more galls in combined treatments.

Urea is readily converted to ammonia by urease present in soil, a conversion that is necessary for urea to be effective both as a fertilizer and as a nematicide. It is generally believed that ammonium form of nitrogen is more damaging to nematodes than nitrate form due to the release of free ammonia into the soil during its decomposition (Badra and Khattab, 1980, Rodriguez-Kábana *et al.*, 1981; Rodriguez-Kábana, 1986). Plasmolytic effect of the urea and its role in the increase of microbiota antagonistic to nematodes have been considered as possible mechanisms of nitrogen fertilizers (Rodriguez-Kábana *et al.*, 1981; Rodriguez-Kábana, 1986). Besides nitrogen, phosphorus is also known to enhance root growth and increase host tolerance along with root absorptive capacity (Hussey and Roncadori, 1982) and to reduce soil pH, which has an adverse effect on nematode multiplication (Pant *et al.*, 1983). Release of fewer root exudates, increase in protein synthesis, cell activity and production of polyphenols, peroxidase and ammonia can be considered as potential mechanisms of the phosphorus effects on plant-parasitic nematodes (Wang and Bergeson, 1974; Marschner, 1997). Potassium had little effect in reducing nematode multiplication in the present study, as it has earlier been also reported (Siddiqui *et al.*, 2001).

Some macronutrient treatments, typically $N_{120}P_{50}K_{25}$ caused a significant increase in root fresh weight. It may be concluded that macronutrient fertilizers are effective for the root system development, and subsequently for the improved plant growth, both in presence or absence of nematode. In general, nutrients can directly or indirectly predispose plants to pathogen attack, because they can reduce or increase disease severity, affect the environment to attract or deter pathogens and also induce resistance or tolerance in the host plant (Santana-Gomes *et al.*, 2013). Integration of macronutrient fertilizers with manures or plant wastes may be a sounder and more effective approach for the control of root-knot and other plant-parasitic nematodes as well as reducing the phytotoxic effect of the chemical fertilizers (Nasr Esfahani and Ahmadi, 2005; Mahmoud *et al.*, 2009).

Similar to the previous surveys (Siddiqui *et al.*, 2001; Nasr Esfahani and Ahmadi, 2005; Noweer and Hasabo, 2005; Parveen *et al.*, 2008; Irshad *et al.*, 2011; Abolusoro *et al.*, 2013), more significant positive effects of N, P and K are evident in the combined application of these fertilizers (*e.g.* in $N_{120}P_{50}K_{12.5}$ and $N_{120}P_{25}K_{25}$) compared to control and use of each macronutrient alone. Single application of nutrients induces several adverse outcomes such as soil salinity, toxic effects to plants and breaking down of plant resistance, but commercial fertilizers usually include a combination of different mineral nutrients and show no or minimal undesirable effects.

Based on our findings, it may be concluded that advisable fertilization formulae for growth improvement of greenhouse cucumber cultivar Negin, infected or non-infected with root-knot nematodes, would be N120-P75-K25 mg/kg of soil in the form of urea, triple super phosphate and potassium sulphate sources, respectively. In a similar study (Charegani *et al.*, 2010), application of 100 mg of nitrogen and 100 mg of phosphorus per kg of soil, also five mg of zinc and 2.5 mg iron per kg of soil caused a significant increase in plant growth parameters, and significant decrease in the number of eggs, egg masses and galls of *M. incognita* in greenhouse cucumber. However, obtained results of these studies under greenhouse condition may be supported by further experiments on other cucumber cultivars, in greenhouse or field conditions to achieveaccepted recommendation to the farmers.

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اثرات برخی عناصر کم‌مصرف و پرمصرف بر روی نماتد ریشه گرهی، *Meloidogyne incognita*، در خیار گلخانه‌ای (*Cucumis sativus* cv. Negin)

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چکیده: اثرات برخی عناصر کم‌مصرف (آهن، روی و سیلیس) و پرمصرف (نیتروژن، فسفر و پتاسیم) بر روی نماتد ریشه گرهی، *Meloidogyne incognita*، و پارامترهای رشد خیار (*Cucumis sativus* cv. Negin) در دو آزمایش مستقل مورد بررسی قرار گرفت. هر یک از عناصر کم‌مصرف آهن، روی و سیلیس به‌میزان پنج میلی‌گرم در کیلوگرم خاک و به‌ترتیب به فرم سکوسترین آهن (Fe-EDDHA)، سولفات روی ($ZnSO_4$) و سیلیکونات سدیم (Na_2O_3Si) مورد استفاده قرار گرفت. هم‌چنین نیتروژن در مقادیر ۶۰، ۱۲۰ و ۱۸۰ میلی‌گرم در کیلوگرم، فسفر در مقادیر ۲۵، ۵۰ و ۷۵ میلی‌گرم در کیلوگرم و پتاسیم در مقادیر ۱۲/۵، ۲۵ و ۳۷/۵ میلی‌گرم در کیلوگرم خاک، به‌ترتیب به فرم اوره، تریپل سوپرفسفات و سولفات پتاسیم مورد استفاده قرار گرفت. تعداد ۸۰۰۰ تخم و لارو سن دوی نماتد (دو عدد تخم و لارو سن دو در گرم خاک) به اطراف ریشه‌ها مایه‌زنی شد. پس از ۶۰ روز، آنالیز داده‌ها نشان داد که تیمار سیلیس+آهن به‌طور معنی‌داری تعداد گال‌های نماتد را در گرم ریشه به‌ترتیب به‌میزان ۵۵ و ۴۲ درصد در دو آزمایش (اول و دوم) کاهش داد، ولی هیچ‌یک از این دو منجر به اثرات مثبت معنی‌دار بر روی افزایش عملکرد رقم مورد بررسی خیار نگردید. نتایج مربوط به عناصر پرمصرف نشان داد که تیمارهای $N_{120}P_{25}K_{25}$ (۱۲۰ میلی‌گرم در کیلوگرم نیتروژن، ۲۵ میلی‌گرم در کیلوگرم فسفر و ۲۵ میلی‌گرم در کیلوگرم پتاسیم) و $N_{120}P_{50}K_{25}$ (۱۲۰ میلی‌گرم در کیلوگرم نیتروژن، ۵۰ میلی‌گرم در کیلوگرم فسفر و ۲۵ میلی‌گرم در کیلوگرم پتاسیم) به‌ترتیب تعداد گال‌های نماتد را به‌میزان ۹۶ و ۸۱ درصد (در آزمایش سوم) و ۷۹ و ۷۰ درصد (در آزمایش چهارم) کاهش می‌دهند. این تیمارها پارامترهای رشد خیار از جمله وزن تر و خشک شاخساره، وزن تر ریشه و وزن میوه را نیز بهبود بخشیدند.

واژگان کلیدی: کنترل، *Meloidogyne incognita*، عناصر کم‌مصرف، عناصر پرمصرف، خیار گلخانه‌ای