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Research Article

# Evaluation of nano iron and zinc chelated fertilizers on okra *Abelmoschus esculentus* infected with *Meloidogyne javanica*

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**Abstract:** Root-knot nematodes, *Meloidogyne* species are among the most critical plant-parasitic nematodes attacking okra *Abelmoschus esculentus* (L.) Moench in tropical and subtropical regions. In the present study, the effects of various levels of zinc and iron on okra infected with *M. javanica* were investigated under greenhouse conditions in a completely randomized factorial design with five replications. Four-leaf stage seedlings of the susceptible okra, cv. Clemson Spineless were inoculated with 8000 eggs of *M. javanica* per pot. Five days later, the soil of each pot was treated with a combination of iron or zinc (0, 1, 3, and 6 mg·kg$^{-1}$ soil) from nano chelated iron and zinc fertilizer sources. Sixty days after inoculation, plants were harvested, and okra growth indices and nematode population indices were determined. Results showed that vegetative indices increased in most cases compared to non-treated plants. Fruit fresh weight of inoculated plants treated with iron at the rate of 1 mg·kg$^{-1}$ soil + zinc at the rate of 3 mg·kg$^{-1}$ soil from nano chelated iron and zinc fertilizer sources increased by 205%, compared to inoculated control plants. Combined application of iron at the rate of 6 mg·kg$^{-1}$ soil + zinc at the rate of 6 mg·kg$^{-1}$ soil from nano chelated iron and zinc fertilizer sources reduced the number of eggs, galls, and egg masses per root system and the reproductive factor (224, 415, 455 and 231%, respectively) compared to non-treated plants.

**Keywords:** Fe and Zn nano chelated fertilizers, management, okra yield, root-knot nematodes

## Introduction

Okra is an important vegetable in tropical and subtropical countries and has high magnesium, potassium, phosphorus, carbohydrates, vitamin A and folic acid (Al-Wandawai, 1983). The total areas under okra cultivation and annual yield production in Iran are 1038 ha and 16791 tons. The major okra growing area in Iran is Khoozestan province, with 16 tons production per hectare (Anonymous, 2019).

Root-knot nematodes *Meloidogyne* species are among the most harmful pathogens on okra *Abelmoschus esculentus* (L.) Moench causing a severe reduction in plant growth and flower formation (Hussain *et al*., 2011; Mukhtar *et al*., 2013). Anwar and McKenry (2012) reported that root-knot nematodes are responsible for yield losses of okra up to 27%. The rapidly growing world population and limitation of food supplies and demand for growing agricultural products have led researchers to seek alternative methods to increase crop yield. One of these methods is the use of pesticides and fertilizers (Zilverberg *et al*., 2010). Organic, chemical, and biological fertilizers are used to improve soil fertility and increase crop yield. The use of fertilizers

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increases yields and possibly reduces damage caused by root-knot nematodes (Ramazani *et al*., 2013). This evidence is that more potent plants have more capacity to compensate for the loss of photosynthesis and reduce the root and leaf area caused by the plant pathogens or stress conditions (Katan, 2009). Okada and Harada (2007) and Fiscus and Neher (2002) showed that fertilizers could affect the population of nematodes in the soil and their effects on crop yield.

Nanotechnology in fertilizer production may lead to optimal release and increase the efficiency of absorption of nutrients in the fertilizer by crops, resulting in significant economic and environmental benefits (Liu and Lal, 2015). Nano fertilizers are nutrient carriers in the range of 30 to 40 nm, and due to their high specific levels, they can carry nutrient ions appropriately (Subramanian *et al*., 2015). Nano fertilizers have high consumption efficiency and can release their nutrients at the right point in the root zone (Zheng *et al*., 2005). Efficient use of nanomaterials in agriculture may complement or replace conventional fertilizers and pesticides, subsequently reducing the environmental impact of agricultural practices. These materials can suppress plant diseases by directly acting on pathogens through various mechanisms, including the generation of reactive oxygen species, and may also suppress disease indirectly by improving crop nutrition and enhancing plant defense pathways (Adisa *et al*., 2019).

This study aimed to determine the effect of nano chelated iron (NCFe), and zinc (NCZn) fertilizers on *M. javanica* infected okra under greenhouse conditions.

## Materials and Methods

### Preparation of nematode

In the summer of 2018, galled roots of tomato plants were collected from the field and greenhouses of Boyer Ahmad County, Iran. Root-knot nematodes were purified using the single egg mass method (Hussey and Barker, 1973), where a single egg mass was removed from galled roots, surface sterilized with 0.5% NaOCl for 2 min rinsed through 3 series of sterilized water. The single egg mass was inoculated to 4 -leaf stage seedlings of susceptible tomato (cv. Early Urbana) and kept at 28 ± 4 °C for three months (Hussey and Barker, 1973). The females of nematode species were identified according to Jepson (1987). More cultures were then produced under the same conditions to increase the inoculum of *M. javanica* required for the experiments (Hartman and Sasser, 1985; Eisenback and Triantaphyllou, 1991).

Tomato roots infected with *M. javanica* were harvested and washed carefully with running water. Roots were chopped into 1 cm pieces ground to fine particles in a 0.5% NaOCl solution using an electric blender for 40 s to extract nematodes eggs. The blender contents were passed through a 74-µm sieve placed on top of a 25-µm sieve and washed immediately with distilled water to remove NaOCl solution. The remaining nematode eggs on the 25µm sieve were washed and transferred to a glass beaker and counted (Hussey and Barker, 1973).

### Preparation of chemical fertilizers

Iron and zinc at the rate of 0, 1, 3, and 6 $mg \cdot kg^{-1}$ soil from NCFe and NCZn sources (Khazra Co., Tehran, Iran) with 12 and 9% purity, respectively, were used. To produce NCZn according to a patent (Nazaran, 2012), 10 g of soluble zinc compound was dissolved in distilled water and put on a shaker to be mixed for 30 min. Afterward, two suitable organic-acid monomers (5 g each) were added to the solution with a pH kept around 6. The solution was blended for 3 h and then 0.5 g of an emulsifying agent added and the mix blended for 15 min at 35 °C. Following that, the solution was left to rest for approximately 1 h and dried at 70 °C. According to patents, the NCFe was produced according to a hydrothermal method (Nazaran, 2012; Fakharzadeh *et al*., 2020).

### Greenhouse studies

Greenhouse experiments were conducted at Boyer-Ahmad County, Iran, in 2019. Plastic





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pots (17.2 cm dia, 17 cm height) were filled with 2 kg steam-sterilized mix of sandy loam soil (pH 7.45, 76% calcium carbonate, 52.9 mg·kg$^{-1}$ phosphorus, 0.170 mg·kg$^{-1}$ of organic matter), sand, and cow manure at a ratio of 1:1:2 by volume, respectively, and seeded with okra cv. Clemson Spineless. Plants were kept in the greenhouse at 28 ± 4 °C with a 16:8 h light to dark photoperiod. Pots were separated into two sets, 4-leaf stage seedlings of the first set inoculated with *M. javanica* eggs (8,000), and the other set was not inoculated (non-inoculated plants). After five days, each set was soil-drenched separately with nano chelated fertilizers, at different levels, with irrigation water. The following treatments were evaluated in each set, including treated plants with iron and zinc 1, 3, 6 mg·kg$^{-1}$ soil separately or in combination with each other, and untreated plants a total of 16 treatments in five replications were performed.

Sixty days after nematode inoculation, plants were harvested, shoot length, shoot and fruit fresh and dry weights, and fresh root weight, as well as the number of eggs, egg masses, and galls per root system, second-stage juveniles (J2s) in soil and the reproduction factor of nematode, were determined (Sasser and Taylor, 1978). One gram of infected root was used for extraction of eggs using 0.5% NaOCl solution. Extracted eggs were counted and converted to the total root volume (Hussey and Barker, 1973). One gram of roots of infected plants using 0.1% fuchsin acid solution was stained (Byrd *et al*., 1983). The numbers of galls and egg masses were counted using a stereomicroscope (40X magnification) and extrapolated to the total root volume. The J2s were extracted from the soil with the Whitehead and Hemming (1965) tray method. The final nematode population was the total number of nematodes in roots and soil, and the reproduction factor was calculated by dividing the final population density of nematode by the initial population density (8000 eggs).

**Statistical analysis**

Data of plant growth parameters were subjected to a 4 × 4 × 2 (NCFe × NCZn × nematode) factorial analysis of variance (ANOVA), and the data of nematode population indices were subjected to a 4 × 4 (NCFe × NCZn) factorial analysis of variance (ANOVA) in a completely randomized design using SAS statistical software ver. 9.4 (ver. 9.4, SAS Institute, Cary, NC). Means were separated using the least significant difference (LSD) test at $P < 0.05$.

## Results

**Impact of nano chelated fertilizers on plant growth parameters**

Shoot length of nematode inoculated plants treated with zinc at the rate of 1 mg·kg$^{-1}$ soil (NCZn1), iron at the rate of 6 mg·kg$^{-1}$ soil (NCFe3), and non-treated plants (control) significantly decreased compared to the non-inoculated plants. Shoot fresh and dry weight of nematode inoculated plants treated with zinc at the rate of 6 mg·kg$^{-1}$ soil (NCZn3) and iron at the rate of 3 mg·kg$^{-1}$ soil + zinc at the rate of 3 mg·kg$^{-1}$ soil (NCFe2 + NCZn2), significantly decreased compared to the non-inoculated plants. Fruit fresh weights of nematode inoculated plants treated with iron at the rate of 3 mg·kg$^{-1}$ soil + zinc at 3 mg·kg$^{-1}$ soil (NCFe2 + NCZn2) significantly decreased non-inoculated plants. Fruit fresh weight of non-inoculated plants treated with iron at the rate of 3 mg·kg$^{-1}$ soil + zinc at 3 mg·kg$^{-1}$ soil (NCFe2 + NCZn2) increased by 165%compared to non-inoculated control plants. Fruit fresh weight of inoculated plants treated with iron at the rate of 1 mg·kg$^{-1}$ soil + zinc at the rate of 3 mg·kg$^{-1}$ soil (NCFe1 + NCZn2) increased by 205%, compared to inoculated control plants. Fruit dry weights of nematode inoculated plants treated with iron at the rate of 1 mg·kg$^{-1}$ soil (NCFe1) significantly decreased compared to the non-inoculated plants. Root fresh weight of nematode inoculated plants treated with iron at the rate of 3 mg·kg$^{-1}$ soil + zinc at 3 mg·kg$^{-1}$ soil (NCFe2 + NCZn2) significantly increased non-inoculated plants (Table 1).





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**Table 1** Mean plant growth parameters of inoculated and non-inoculated okra plants with *Meloidogyne javanica* treated with nano chelated iron (NCFe) and zinc (NCZn) fertilizer.

| Fe (mg·kg$^{-1}$ soil) | Zn (mg·kg$^{-1}$ soil) | Nematode | Shoot length (cm) | Shoot fresh weight (g) | Shoot dry weight (g) | Root fresh weight (g) | fruit fresh weight (g) | fruit dry weight (g) |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | NI | $60.4 \pm 0.9^{b-h}$ | $16.0 \pm 0.2^{e-l}$ | $3.0 \pm 0.2^{c-h}$ | $5.1 \pm 0.5^{a-e}$ | $6.2 \pm 0.6^{bcd}$ | $3.1 \pm 0.3^{b-i}$ |
| | | I | $49.6 \pm 0.5^{i-j}$ | $11.2 \pm 0.4^{klm}$ | $3.0 \pm 0.1^{b-h}$ | $5.0 \pm 0.2^{a-e}$ | $4.0 \pm 0.8^{cd}$ | $1.7 \pm 0.1^{h-i}$ |
| | 1 | NI | $62.9 \pm 1.5^{a-g}$ | $14.4 \pm 0.5^{f-m}$ | $4.7 \pm 0.3^{a-h}$ | $6.1 \pm 0.6^{a-d}$ | $8.9 \pm 1^{a-d}$ | $4.2 \pm 0.2^{abc}$ |
| | | I | $52.5 \pm 1.6^{hij}$ | $12.6 \pm 0.1^{h-l}$ | $2.9 \pm 0.4^{c-h}$ | $3.4 \pm 0.6^{de}$ | $8.5 \pm 0.8^{a-d}$ | $3.1 \pm 0.1^{b-i}$ |
| | 3 | NI | $63.8 \pm 1^{a-g}$ | $19.6 \pm 0.1^{a-g}$ | $4.7 \pm 0.4^{a-g}$ | $5.2 \pm 0.4^{a-e}$ | $11.2 \pm 1.6^{abc}$ | $3.1 \pm 0.2^{b-i}$ |
| | | I | $56.2 \pm 0.8^{c-j}$ | $13.0 \pm 0.4^{g-m}$ | $3.4 \pm 0.3^{a-h}$ | $3.7 \pm 0.5^{cde}$ | $5.1 \pm 1.6^{bcd}$ | $2.8 \pm 0.1^{c-i}$ |
| | 6 | NI | $60.9 \pm 1.2^{b-h}$ | $19.7 \pm 0.3^{a-g}$ | $5.4 \pm 0.2^{ab}$ | $5.5 \pm 0.8^{a-e}$ | $9.5 \pm 0.9^{a-d}$ | $2.4 \pm 0.3^{d-i}$ |
| | | I | $54.8 \pm 0.9^{e-j}$ | $9.7 \pm 0.6^{m}$ | $2.362 \pm 0.2^{gh}$ | $3.4 \pm 0.7^{de}$ | $7.4 \pm 1.3^{bcd}$ | $2.6 \pm 0.2^{c-i}$ |
| 1 | 0 | NI | $63.2 \pm 0.8^{a-g}$ | $17.3 \pm 0.4^{d-k}$ | $4.8 \pm 0.2^{a-f}$ | $4.6 \pm 0.6^{a-e}$ | $13.6 \pm 2.1^{ab}$ | $5.1 \pm 0.1^{a}$ |
| | | I | $65.3 \pm 1.9^{a-f}$ | $15.7 \pm 0.4^{e-m}$ | $3.1 \pm 0.2^{b-h}$ | $5.1 \pm 0.7^{a-e}$ | $7.2 \pm 1.9^{bcd}$ | $2.6 \pm 0.1^{c-i}$ |
| | 1 | NI | $62.1 \pm 1.3^{b-h}$ | $24.1 \pm 0.2^{abc}$ | $4.9 \pm 0.3^{a-d}$ | $4.9 \pm 0.8^{a-e}$ | $7.9 \pm 1.6^{bcd}$ | $3.2 \pm 0.1^{b-i}$ |
| | | I | $59.9 \pm 1.5^{c-h}$ | $13.5 \pm 0.1^{g-m}$ | $3.1 \pm 0.2^{b-h}$ | $4.7 \pm 0.5^{a-e}$ | $5.0 \pm 1.2^{cd}$ | $1.6 \pm 0.2^{i}$ |
| | 3 | NI | $68.8 \pm 1.6^{a-d}$ | $25.5 \pm 0.6^{a}$ | $5 \pm 0.3^{a-d}$ | $5.8 \pm 0.4^{a-e}$ | $12.8 \pm 1.5^{abc}$ | $4.5 \pm 0.2^{ab}$ |
| | | I | $70.5 \pm 1.1^{ab}$ | $25.8 \pm 1^{a}$ | $4.9 \pm 0.2^{a-e}$ | $6.9 \pm 0.4^{ab}$ | $12.2 \pm 1.6^{abc}$ | $3.5 \pm 0.1^{b-f}$ |
| | 6 | NI | $66.5 \pm 1^{a-d}$ | $24.8 \pm 0.3^{ab}$ | $4.8 \pm 0.3^{a-f}$ | $5.3 \pm 0.6^{a-e}$ | $8.2 \pm 1.5^{a-d}$ | $3.5 \pm 0.3^{b-g}$ |
| | | I | $67.3 \pm 1.4^{a-d}$ | $17.0 \pm 0.7^{d-k}$ | $4.3 \pm 0.3^{a-h}$ | $5.7 \pm 0.8^{a-e}$ | $5.8 \pm 1^{bcd}$ | $1.9 \pm 0.2^{ghi}$ |
| 3 | 0 | NI | $69.6 \pm 1.3^{abc}$ | $21.5 \pm 0.4^{a-e}$ | $5.0 \pm 0.2^{a-d}$ | $4.6 \pm 0.8^{a-e}$ | $12.6 \pm 1.1^{abc}$ | $3.5 \pm 0.2^{b-f}$ |
| | | I | $61.0 \pm 1.5^{b-h}$ | $14.0 \pm 0.5^{f-m}$ | $3.4 \pm 0.4^{a-h}$ | $7.2 \pm 0.3^{a}$ | $11.4 \pm 1.1^{abc}$ | $3.4 \pm 0.1^{b-g}$ |
| | 1 | NI | $72.9 \pm 1.9^{a}$ | $20.4 \pm 0.5^{a-f}$ | $4.3 \pm 0.3^{a-h}$ | $3.9 \pm 0.7^{cde}$ | $12.2 \pm 0.8^{abc}$ | $3.8 \pm 0.1^{a-e}$ |
| | | I | $66.5 \pm 2^{a-d}$ | $18.6 \pm 0.4^{b-i}$ | $3.5 \pm 0.2^{a-h}$ | $6.5 \pm 0.4^{abc}$ | $10.6 \pm 0.9^{a-d}$ | $3.8 \pm 0.2^{a-d}$ |
| | 3 | NI | $68.7 \pm 2.1^{a-d}$ | $23.1 \pm 0.2^{a-d}$ | $5.2 \pm 0.5^{abc}$ | $3.3 \pm 0.4^{e}$ | $16.4 \pm 1.6^{a}$ | $3.5 \pm 0.2^{b-g}$ |
| | | I | $58.8 \pm 2.6^{d-i}$ | $12.0 \pm 0.3^{i-m}$ | $2.4 \pm 0.3^{fgh}$ | $6.5 \pm 0.6^{abc}$ | $7.5 \pm 1.3^{bcd}$ | $2.7 \pm 0.1^{c-i}$ |
| | 6 | NI | $65.5 \pm 0.7^{a-f}$ | $20.4 \pm 0.6^{a-f}$ | $4.3 \pm 0.3^{a-h}$ | $4.2 \pm 0.4^{b-e}$ | $8.7 \pm 1.3^{a-d}$ | $2.8 \pm 0.2^{c-i}$ |
| | | I | $55.3 \pm 2.1^{f-j}$ | $13.0 \pm 0.6^{g-m}$ | $3.8 \pm 0.2^{a-h}$ | $5.6 \pm 0.5^{a-e}$ | $7.5 \pm 1^{bcd}$ | $2.3 \pm 0.1^{d-i}$ |
| 6 | 0 | NI | $63.8 \pm 1.1^{a-g}$ | $15.0 \pm 0.5^{f-m}$ | $3 \pm 0.2^{b-h}$ | $4.3 \pm 0.3^{b-e}$ | $6.3 \pm 1^{bcd}$ | $2.2 \pm 0.1^{e-i}$ |
| | | I | $48.1 \pm 1.8^{j}$ | $10.9 \pm 0.8^{klm}$ | $2.3 \pm 0.2^{h}$ | $3.8 \pm 0.3^{cde}$ | $4.9 \pm 1.2^{cd}$ | $2.1 \pm 0.1^{f-i}$ |
| | 1 | NI | $66.1 \pm 1.2^{a-e}$ | $20.2 \pm 0.1^{a-f}$ | $5.6 \pm 0.5^{a}$ | $6.1 \pm 0.7^{a-e}$ | $10.8 \pm 1.5^{abc}$ | $4.2 \pm 0.4^{abc}$ |
| | | I | $60.5 \pm 2^{b-h}$ | $16.6 \pm 0.2^{d-k}$ | $3.7 \pm 0.4^{a-h}$ | $6.1 \pm 0.3^{a-e}$ | $5.8 \pm 1.6^{bcd}$ | $2.6 \pm 0.1^{c-i}$ |
| | 3 | NI | $66.3 \pm 0.6^{a-e}$ | $18.0 \pm 0.2^{c-j}$ | $3.9 \pm 0.3^{a-h}$ | $4.6 \pm 0.8^{a-e}$ | $9.7 \pm 0.8^{a-d}$ | $3.2 \pm 0.2^{b-h}$ |
| | | I | $67.0 \pm 2.6^{a-d}$ | $17.9 \pm 0.6^{c-j}$ | $4.6 \pm 0.5^{a-h}$ | $5.5 \pm 0.9^{a-e}$ | $8.5 \pm 1^{a-d}$ | $2.9 \pm 0.1^{c-i}$ |
| | 6 | NI | $59.1 \pm 1.6^{d-i}$ | $12.7 \pm 0.4^{h-m}$ | $2.7 \pm 0.1^{d-h}$ | $4.4 \pm 0.3^{a-e}$ | $8.2 \pm 1.1^{a-d}$ | $3.0 \pm 0.2^{b-i}$ |
| | | I | $55.4 \pm 2.7^{f-j}$ | $11.3 \pm 0.5^{j-m}$ | $2.8 \pm 0.2^{c-h}$ | $3.9 \pm 0.4^{cde}$ | $7.8 \pm 1.6^{bcd}$ | $2.7 \pm 0.1^{c-i}$ |

NI: Non-inoculated, I; Inoculated.
Values are mean ± standard error of the mean with five replicates. Values in each column followed by different letters are significantly different according to the least significant difference (LSD) test at $P < 0.05$.

**Impact of nano chelated fertilizers on nematode indices**

The reproduction factor of nematode and numbers of eggs per root system in treated plants with zinc at the rate of 1 mg·kg$^{-1}$ soil (NCZn1) and 6 mg·kg$^{-1}$ soil (NCZn3) as well as treated plants with iron at the rate of 6 mg·kg$^{-1}$ soil + zinc at the rate of 6 mg·kg$^{-1}$ soil (NCFe3 + NCZn3), significantly decreased compared with control (NCFe0 + NCZn0). The nematode reproduction factor on the treated okra plants with iron at the rate of 3 mg·kg$^{-1}$ soil + zinc at 3 mg·kg$^{-1}$ soil (NCFe3 + NCZn3) decreased by 231% control plants.

Some treatments significantly decreased the number of galls and egg masses per root system compared with control. The highest numbers of J2s in soil were observed in the treated plants with iron at the rate of 3 mg·kg$^{-1}$ soil (NCFe2) (Table 2).





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**Table 2** Mean nematode population indices on okra plants inoculated with *Meloidogyne javanica* and treated with 0, 1, 3, and 6 mg·kg$^{-1}$ soil of zinc and iron from nano chelated iron (NcFe) and zinc (NcZn) fertilizer sources 60 days after nematode inoculation.

| Fe (mg·kg$^{-1}$ soil) | Zn (mg·kg$^{-1}$ soil) | No. eggs (root system)$^{-1}$ | No. galls (root system)$^{-1}$ | No. egg masses (root system)$^{-1}$ | No. J2s (2000 cm$^3$)$^{-1}$ | Reproduction factor |
|---|---|---|---|---|---|---|
| 0 | 0 | 29818 ± 3560$^{def}$ | 392 ± 25$^{bc}$ | 367 ± 42$^{bc}$ | 995 ± 165$^{de}$ | 5.2 ± 0.6$^{de}$ |
| | 1 | 11681 ± 2140$^{gh}$ | 129 ± 24$^{ghi}$ | 92 ± 34$^{gh}$ | 885 ± 190$^{de}$ | 2.1 ± 0.5$^{fg}$ |
| | 3 | 19019 ± 2500$^{fgh}$ | 281 ± 46$^{def}$ | 262 ± 42$^{de}$ | 1678 ± 241$^{cde}$ | 3.6 ± 0.6$^{efg}$ |
| | 6 | 8796 ± 2640$^{gh}$ | 103 ± 36$^{hi}$ | 85 ± 46$^{gh}$ | 1185 ± 203$^{de}$ | 1.6 ± 0.3$^{g}$ |
| 1 | 0 | 28700 ± 4800$^{def}$ | 112 ± 30$^{ghi}$ | 82 ± 41$^{gh}$ | 625 ± 165$^{de}$ | 5.2 ± 0.8$^{de}$ |
| | 1 | 40263 ± 6900$^{cde}$ | 186 ± 38$^{fgh}$ | 145 ± 29$^{fgh}$ | 171 ± 62$^{e}$ | 6.8 ± 1$^{cd}$ |
| | 3 | 69730 ± 6480$^{a}$ | 213 ± 42$^{efg}$ | 174 ± 30$^{efg}$ | 3715 ± 456$^{b}$ | 12.3 ± 1.1$^{a}$ |
| | 6 | 57919 ± 5880$^{ab}$ | 517 ± 56$^{a}$ | 462 ± 43$^{a}$ | 750 ± 150$^{de}$ | 9.8 ± 1.3$^{ab}$ |
| 3 | 0 | 48569 ± 4210$^{bc}$ | 383 ± 41$^{bc}$ | 315 ± 44$^{bcd}$ | 8615 ± 660$^{a}$ | 10.6 ± 1.2$^{ab}$ |
| | 1 | 28837 ± 3900$^{def}$ | 277 ± 39$^{def}$ | 229 ± 42$^{def}$ | 450 ± 160$^{de}$ | 4.9 ± 1$^{de}$ |
| | 3 | 37605 ± 4500$^{cde}$ | 149 ± 22$^{ghi}$ | 166 ± 38$^{e-h}$ | 440 ± 188$^{de}$ | 6.4 ± 0.9$^{cd}$ |
| | 6 | 41160 ± 4440$^{cd}$ | 441 ± 40$^{ab}$ | 363 ± 35$^{bc}$ | 650 ± 220$^{de}$ | 8.4 ± 1$^{bc}$ |
| 6 | 0 | 23364 ± 5200$^{efg}$ | 348 ± 48$^{bcd}$ | 280 ± 36$^{cd}$ | 1495 ± 350$^{cde}$ | 4.2 ± 1.1$^{def}$ |
| | 1 | 17661 ± 5010$^{fgh}$ | 302 ± 51$^{cde}$ | 262 ± 30$^{de}$ | 3320 ± 650$^{bc}$ | 3.7 ± 0.9$^{efg}$ |
| | 3 | 24000 ± 4800$^{efg}$ | 498 ± 34$^{a}$ | 412 ± 41$^{ab}$ | 2425 ± 620$^{bcd}$ | 4.3 ± 0.8$^{def}$ |
| | 6 | 9203 ± 3650$^{gh}$ | 76 ± 26$^{i}$ | 66 ± 16$^{h}$ | 150 ± 50$^{e}$ | 1.6 ± 0.6$^{g}$ |

Values are mean ± standard error of the mean with five replicates. Values in each column followed by different letters are significantly different according to the least significant difference (LSD) test at $P < 0.05$. J2s = second-stage juveniles.

## Discussion

It is known that micronutrients are fundamental in plant metabolism, acting on the phenol and lignin content and membrane stability (Marschner, 2012). Previous studies showed that iron is essential for many physiological and biochemical processes, including the production of chlorophyll, oxidation-reduction reactions, photosynthesis, respiration, and enzymatic systems. Although the total amount of this element is high in the soil, some of the chemical and physical properties of soils, such as alkaline pH, organic matter shortage, excessive use of phosphorus fertilizers, and iron nutrition by plants, reduce iron availability to plants (Abd El-Wahab, 2008; Marschner, 2012). Various kinds of iron compounds are used worldwide. Iron chelates have shown a higher absorption rate compared to other iron fertilizers (Tyksiński, 2008). However, iron chelates have some limitations in application and absorption rate (Karagiannidis *et al*., 2008).

In the present study, the effect of zinc and iron as nano chelated fertilizers was investigated on the vegetative parameters of okra and population indices of *M. javanica*. We showed that the combined application of iron and zinc at the rate of 6 mg·kg$^{-1}$ soil from nano chelated fertilizer sources reduced the number of eggs, galls, and egg masses per root system and the reproductive factor of *M. javanica* on okra plants, compared to non-treated plants. The efficiency of nano chelated fertilizers that were synthesized based on nano chelating technology was proved. Zareabyaneh and Bayatvarkeshi indicated that nano-nitrogen chelate and sulfur-coated nano-nitrogen chelate significantly affected potato yield, leaching, and soil nitrate (Zareabyaneh and Bayatvarkeshi, 2015). It was revealed that sprayings on apple trees with nano calcium fertilizer increased firmness, titrable acidity, total phenolic content, browning, total antioxidant activity, and fiber content in apple fruit, compared to control fruit (Ranjbar *et al*., 2018).

Applying nano chelated fertilizers improved all vegetative parameters of plants in the current study. Other researches with nano chelated fertilizers have demonstrated similar results. Ghasemi Lemraski *et al*. (2017) showed that



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rice tiller number, panicle length, paddy yield, and plant height were positively affected by nano chelated iron fertilizer application. Fakharzadeh *et al*. (2020) revealed that the application of nano chelated iron fertilizer enhanced protein concentration and nitrogen, phosphorus, and potassium content of rice. It shows that applying nano chelated iron fertilizer increases the uptake of macronutrients and decreases the need for chemical fertilizers.

In the present study, nematode inoculated plants non-treated with fertilizers (inoculated control) had the lowest fruit fresh weight and shoot length than the other treated plants. It revealed that *M. javanica* harmed okra plants, but the single and combined application of iron and zinc from nano chelated fertilizer sources reduced these adverse effects. It is further proved that root-knot nematodes decrease stomatal conductivity, transpiration, and photosynthesis (Strajnar *et al*., 2012) and suppress water flow through intact roots, leading to severe drought stress in plants (Alzarqaa *et al*., 2014). Previous studies have shown that the application of nano chelated iron fertilizer positively affects growth indices of *Calendula officinalis* under water stress conditions (Pirzad and Shokrani, 2012). Therefore, this fertilizer can reduce the effect of drought stress caused by nematodes.

Li *et al*. (2006) stated that zinc is considered a vital compound in enzymes, including glutamate dehydrogenase, catalase, and superoxide dismutase, and plays a role in the synthesis of chlorophyll, indole 3-acetic acid, and proteins. The plants with zinc deficiency contain low levels of superoxide dismutase, resulting in high levels of superoxide radicals, increased peroxidation of membrane lipid, and membrane destruction, which increases the permeability of the membrane (Cakmak and Marschner, 1988). Root exudates attract nematodes to the roots. Root oxidation is higher in plants with a shortage of zinc and causes the attraction of these parasites, and thus the rate of disease and infection increases (Streeter *et al*., 2001). Zinc is one of the necessary micro-nutrients that act as a critical ingredient in the structure of many enzymes. Zinc is considered an essential element in dehydrogenase, proteinase, RNA, and growth regulators. Zinc is adequate on many critical biochemical pathways associated with carbohydrate metabolism (including photosynthesis and conversion of sugars to starch), protein metabolism, auxin metabolism, cell membrane integrity, and resistance to pathogens (Alloway, 2008). Generally, zinc is a critical element in the metabolism of proteins and enzymes and photosynthetic pigments that can increase the photosynthetic potential and crop yield. However, excess of this element can reduce root and shoot growth, absorb other nutrients, especially phosphorus, and increase excessive iron absorption (Rosen *et al*., 1977).

The present study revealed that zinc fertilizers are effective on vegetative parameters of okra plants, which agrees with previous studies. Rezaei and Abbasi (2014) reported that the application of chelated and nano chelated zinc fertilizers on cotton plants improved chlorophyll, increased the height and fresh and dry weight of plants, and increased antioxidant activity peroxidase, catalase, and polyphenol oxidase. As previously reported, peroxidase and polyphenol oxidase catalyzes the last step in lignin biosynthesis and other oxidative phenols. Enhanced activity of this antioxidant is inversely related to the reproduction rate of the root-knot nematodes (Sahebani *et al*., 2011).

In the current study, combined application of iron at the rate of 6 $mg \cdot kg^{-1}$ soil + zinc at the rate of 6 $mg \cdot kg^{-1}$ soil decreased the reproduction of *M. javanica* compared to non-treated plants. This result agrees with previous reports that showed the positive effect of fertilizers against the root-knot nematodes. Oka and Pivonia (2002) showed that the use of organic and chemical fertilizers has an inhibiting effect on root-knot nematode and other plant-parasitic nematodes. The reproduction factor of *M. javanica* on treated eggplants with nitrogen at the rate of 100 $mg \cdot kg^{-1}$ soil + phosphorus at the rate of 100 $mg \cdot kg^{-1}$ soil from nano chelated nitrogen fertilizer, and nano chelated phosphorus fertilizer decreased by 55%, compared to control plants (Mozaffarian *et*





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*al*., 2019). Reduction of *M. incognita* population and improvement of cucumber growth indices were proved by the application of sodium silicate (Na2O3Si) alone and in combination with ethylenediamine di-2-hydroxyphenyl acetate ferric (Fe-EDDHA) and zinc sulfate (ZnSO4) (Ahmadi Mansourabad *et al*., 2016). In a study, it was found that the combination of nitrogen, phosphorus, potassium, and zinc reduced the damage of *M. incognita* in okra and also improved plant growth indices compared to the control plants (Bamel *et al*., 2003). Fertilizing okra plants with nitrogen and phosphorus (100 and 25 mg/10,000 $m^2$ soil, respectively) reduced the number of eggs of *M. javanica* (Verma and Gupta, 1987). Infected okra by *M. javanica* treated with two widely used fertilizers (urea and NPK at the rate of 0.1%) showed a significant increase of plant growth indices and reduced the number of galls compared to control (Irshad *et al*., 2006). In a study by Gkanatsiou *et al*. (2019), it was demonstrated that using copper/iron nanoparticles significantly reduced the number of galls and females of root-knot nematodes in tomato roots.

Using nano chelated fertilizers to increase crop yield and other disease control methods instead of using pesticides can be economical. Chemical pesticides have severe effects on soil ecology that may lead to alterations or the erosion of beneficial or plant probiotic soil microflora. In conclusion, owing to minimal quantities of nano chelated fertilizers and the positive effect of iron and zinc on the plant's vegetative growth and their inhibitory effects on *M. javanica*, they can be used as an alternative to commercial nematicides for the management of plant-parasitic nematodes.

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**Conflict of interest**
The authors declare no conflict of interest.

**References**

Abd El-Wahab, M. A. 2008. Effect of some trace elements on growth, yield and chemical constituents of *Trachyspermum ammi*. L. (AJOWAN) plants under Sinai conditions. Research Journal of Agriculture and Biological Sciences, 4(6): 717-724.

Adisa, I. O., Pullagurala, V. L., Peralta-Videa, J. R., Dimkpa, C. O., Elmer, W. H., Gardea-Torresdey, J. and White, J. 2019. Recent advances in nano-enabled fertilizers and pesticides: A critical review of mechanisms of action. Environmental Science: Nano, 6:2002-2030.

Ahmadi Mansourabad, M., Kargar Bideh, A. and Abdollahi, M. 2016. Effects of some micronutrients and macronutrients on the root-knot nematode, *Meloidogyne incognita*, in greenhouse cucumber (*Cucumis sativus* cv. Negin). Journal of Crop Protection, 5 (4): 507-517.

Alloway, B. J. 2008. Zinc in soils and crop nutrition, 2nd Ed. IZA and IFA Publication, Brussels, Belgium and Paris, France.

Al-Wandawai, H. 1983. Chemical composition of seeds of two okra cultivars. Journal of Agricultural and Food Chemistry, 31: 1355-1358.

Alzarqaa, A. A., Roushdy, S. S., Alderfasi, A. A., Al-Yahya, F. A. and Dawabah A. A. M. 2014. The physiological response of mungbean (*Vigna radiata*) to water deficit stress and *Meloidogyne javanica* infection. Sustainable Irrigation and Drainage, 185: 89-100.

Anonymous, 2019. Agricultural Statistics of Iran. Iran Ministry of Agriculture-Jihad. Department of Planning and Economy, Information and Communication Technology Center.

Anwar, S. A. and McKenry, M. V. 2012. Incidence and population density of plant parasitic nematodes infecting vegetable crops and associated yield losses. Pakistan Journal of Zoology, 44: 327-333.

Bamel, V., Verma, K. K. and Gupta, D. C. 2003. Effect of different fertilizer source on





[ DOR: 20.1001.1.22519041.2021.10.3.2.0 ]

*Meloidogyne incognita* infecting okra (*Abelmoschus esculentus*). Indian Journal of Nematology, 33: 132-135.

Byrd, D. W., Kirkpatrick, T. and Barker, K. R. 1983. An improved technique for clearing and staining plant tissue for detection of nematodes. Journal of Nematology, 15: 142-143.

Cakmak, I. and Marschner, H. 1988. Enhanced superoxide radical production in roots of zinc-deficient plants. Journal of Experimental Botany, 39: 1449-1460.

Eisenback, J. D. and Triantaphyllou, H. H. 1991. Root-knot nematodes: *Meloidogyne* species and races. Manual of Agricultural Nematology, 1: 191-274.

Fakharzadeh, S., Hafizi, M., Baghaei, M. A., Etesami, M., Khayamzadeh, M., Kalanaky, S., Akbari, M. E. and Nazaran, M. H. 2020. Using Nanochelating technology for biofortification and yield increase in rice. Scientific Reports, 10: 4351. https://doi.org/10.1038/s41598-020-60189-x.

Fiscus, D. A. and Neher, D. A. 2002. Distinguishing sensitivity of free-living soil nematode genera to physical and chemical disturbances. Ecological Applications, 12: 565-575.

Ghasemi Lemraski, M., Normohamadi, G., Madani, H., Heidari Sharif Abad, H. and Mobasser, H. R. 2017. Two Iranian rice cultivars' response to nitrogen and nano fertilizer. Open Journal of Ecology, 7: 591-603.

Gkanatsiou, C., Ntalli, N., Menkissoglu-Spiroudi, U. and Dendrinou-Samara, C. 2019. Essential metal-based nanoparticles (copper/iron NPs) as potent nematicidal agents against *Meloidogyne* spp. Journal of Nanoparticle Research, 1: 44-58.

Hartman, K. M. and Sassre, J. N. 1985. Identification of *Meloidogyne* species on the basis of differential host and perineal-pattern morphology, In: Barker, K. R., Carter, C. C. and Sasser, J. N. (Eds.), An Advanced Treatise on *Meloidogyne*. North Carolina State University Graphics, Raleigh, NC. pp. 69-77.

Hussain, M. A., Mukhtar, T. and Kayani, M. Z. 2011. Assessment of the damage caused by *Meloidogyne incognita* on Okra (*Abelmoschus esculentus*). Journal of Animal and Plant Sciences, 21: 857-861.

Hussey, R. S. and Barker, K. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter, 57: 1025-1028.

Irshad, L., Dawar, S., Zaki, M. J. and Ghaffar, A. 2006. Effect of nursery fertilizers on plant growth and the control of *Meloidogyne javanica* root-knot nematode on mung bean and okra plants. Pakistan Journal of Botany, 38: 1301-1304.

Jepson, S. B. 1987. Identification of root-knot nematode (*Meloidogyne*) species. CAB International, Wallingford, UK.

Karagiannidis, N., Thomidis, T., Zakinthinos, G. and Tsipouridis, C. 2008. Prognosis and correction of iron chlorosis in peach trees and relationship between iron concentration and Brown Rot. Scientia Horticulturae, 118: 212-217.

Katan, J. 2009. Mineral nutrient management and plant disease. International Fertilizer Correspondent, 21: 6-8.

Li, W. Y. F., Wong, F. L. Tsai, S. N., Phang, T. H., Shao, G. H. and Lam, H. M. 2006. Tonoplastlocated GmCLC1 and GmNHX1 from soybean enhance NaCl tolerance in transgenic bright yellow (by)-2 Cells. Plant Cell Environment, 29: 1122-1137.

Liu, R. and Lal, R. 2015. Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. A Review. Science of the Total Environment, 514: 131-139.

Marschner, P. 2012. Mineral Nutrition of Higher Plants. 3. ed. London: Academic Press.

Mozaffarian, S., Charehgani, H., Abdollahi, M. and Rezaei, R. 2019. Effect of some macronutrients on the root-knot nematode, *Meloidogyne javanica* activities in eggplant (*Solanum melongena* L.). Journal of Plant Protection, 33: 397-408.

Mukhtar, T., Kayani, M. Z. and Hussain, M. A. 2013. Nematicidal activities of *Cannabis*





[ DOR: 20.1001.1.22519041.2021.10.3.2.0 ]

*sativa* L. and *Zanthoxylum alatum* Roxb. against *Meloidogyne incognita*. Industrial Crops and Products, 42: 447-453.

Nazaran, M. H. 2012. Chelate compounds. Google Patents, US8288587B2.

Oka, Y. and Pivonia, S. 2002. Use of ammonia-releasing compounds for control of the root-knot nematode *Meloidogyne javanica*. Nematology, 4: 65-71.

Okada, H. and Harada, H. 2007. Effects of tillage and fertilizer on nematode communities in a Japanese soybean field. Applied Soil Ecology, 35: 528-598.

Pirzad, A. and Shokrani, F. 2012. Effects of iron application on growth characters and flower yield of *Calendula officinalis* L. under water stress. World Applied Sciences Journal, 18: 1203-1208.

Ramazani, B. E., Mahdikhani, M. and Rohani, H. 2013. Evaluation of resistance of some tomato cultivars to root-knot nematode (*Meloidogyne javanica*) in greenhouse condition. Journal of Plant Protection, 27: 276-285.

Ranjbar, S., Rahemi, M. and Ramezanian, A. 2018. Comparison of nano-calcium and calcium chloride spray on postharvest quality and cell wall enzymes activity in apple cv. Red Delicious. Scientia Horticulturae, 240: 57-64.

Rezaei, M. and Abbasi, H. 2014. Foliar application of nano-chelate and non-nanochelate of zinc on plant resistance physiological processes in cotton (*Gossipium hirsutum* L.). Iranian Journal of Plant Physiology, (4): 1137-1144.

Rosen, J. A., Pike, C. S. and Golden, M. L. 1977. Zinc, iron and chlorophyll metabolism in Zinc-toxic corn. Plant Physiology, 59: 1085-1087.

Sahebani, N., Hadavi, N. and Omran Zade, F. 2011. The effects of b-amino-butyric acid on resistance of cucumber against root-knot nematode, *Meloidogyne javanica*. Acta Physiologiae Plantarum, 33: 443-450.

Sasser, J. N. and Taylor, A. L. 1978. Biology, identification and control of root knot nematodes (*Meloidogyne* spp.). NC, USA: North Carolina State Graphics, Raleigh.

Strajnar, P., Sirca, S., Urek, G., Sircelj, H., Zeleznik, P. and Vodnik, D. 2012. Effect of *Meloidogyne ethiopica* parasitism on water management and physiological stress in tomato. European Journal of Plant Pathology, 132: 49-57.

Streeter, T. C., Rengel, Z., Neate, S. M. and Graham, R. D. 2001. Zinc fertilization increases tolerance to *Rhizoctonia solani* (AG 8) in *Medicago truncatula*. Plant and Soil, 228: 233-242.

Subramanian, S., Blanton, L. V., Frese, S. A., Charbonneau, M., Mills, D. A. and Gordon, J. I. 2015. Cultivating healthy growth and nutrition through the gut microbiota. Cell, 161(1): 36-48.

Tyksiński, W. K. A. 2008. After effect of iron chelates on the yielding and iron content in greenhouse lettuce. Acta Scientiarum Polonorum Hortorum Cultus, 7: 3-10.

Verma, K. K. and Gupta, D. C. 1987. Effect of NPK fertilizers alone and in combination with aldicarb and carbofuran on okra infected with *Meloidogyne javanica*. Indian Journal of Agricultural Science, 57: 609-611.

Whitehead, A. G. and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology, 55: 25-38.

Zareabyaneh, H. and Bayatvarkeshi, M. 2015. Effects of slow-release fertilizers on nitrate leaching, its distribution in soil profile, N-use efficiency, and yield in potato crop. Environmental Earth Sciences, 74: 3385-3393.

Zheng, L., Hong, F., Lu, S. and Liu, C. 2005. Effect of nano-$TiO_2$ on strength of naturally aged seeds and growth of spinach. Biological Trace Element Research, 104: 163-168.

Zilverberg, C., Kreuter, U. and Conner, R. 2010. Population growth and fertilizer use: ecological and economic consequences in Santa Cruz del Quiché, Guatemala. Society and Natural Resources, 23: 1-13.





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# ارزیابی نانوکودهای کلات روی و آهن در گیاهان بامیه *Abelmoschus esculentus* آلوده به نماتد *Meloidogyne javanica*

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**چکیده:** نماتدهای ریشه‌گرهی *Meloidogyne* spp. یکی از مهم‌ترین نماتدهای انگل گیاهی در گیاه بامیه *Abelmoschus esculentus* (L.) Moench در مناطق استوایی و نیمه‌استوایی می‌باشند. در مطالعه حاضر، تأثیر سطوح مختلف روی و آهن در گیاه بامیه آلوده به نماتد *M. javanica* در شرایط گلخانه به‌صورت فاکتوریل در قالب طرح کاملاً تصادفی با پنج تکرار ارزیابی شد. گیاهچه‌های بامیه رقم حساس Clemson Spineless با ۸۰۰۰ تخم نماتد *M. javanica* در گلدان مایه‌زنی شد. پس از گذشت پنج روز، خاک هر گلدان با ترکیبی از غلظت‌های صفر (شاهد)، ۱، ۳ و ۶ میلی‌گرم روی و آهن در کیلوگرم خاک از منابع نانوکود کلات روی و نانوکود کلات آهن تیمار شدند. پس از گذشت ۶۰ روز، گیاهان برداشت و شاخص‌های رویشی بامیه و شاخص‌های جمعیتی نماتد ارزیابی شدند. نتایج نشان داد که شاخص‌های رویشی گیاه در اغلب تیمارها در مقایسه با گیاهان شاهد افزایش یافت. در گیاهان آلوده و تیمار شده با ۱ میلی‌گرم آهن + ۳ میلی‌گرم روی از منابع نانوکود کلات آهن و نانوکود کلات روی در مقایسه با گیاهان شاهد، وزن تر میوه به‌میزان ۲۰۵ درصد افزایش یافت. در گیاهان تیمار شده با تلفیق ۶ میلی‌گرم آهن + ۶ میلی‌گرم روی از منابع نانوکود کلات آهن و نانوکود کلات روی در مقایسه با گیاهان شاهد، به‌ترتیب کاهش ۲۲۴، ۴۱۵، ۴۵۵ و ۲۳۱ درصدی در تعداد تخم، گال و کیسه تخم در سیستم ریشه و فاکتور تولیدمثل نماتد مشاهده شد.

**واژگان کلیدی:** نانوکود کلات آهن و روی، مدیریت، محصول بامیه، نماتد ریشه‌گرهی



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