EFFECTS OF HIGH NICKEL SOIL ON
ROOT-KNOT NEMATODE DISEASE OF TOMATO

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ABSTRACT

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High nickel (Ni) soils are becoming increasingly common where soils are exposed to waste products, and can be harmful to agricultural enterprises. In a study of tomato (*Lycopersicon esculentum* cv. Pusa Ruby) plants exposed to high soil Ni and nematodes, it was found that both interact to affect growth of young plants. Soils amended with Ni at 0, 10, 50, 100, 200 and 400 mg/L and subsequently inoculated with 2000 juveniles of *Meloidogyne incognita/*pot resulted in substantial growth reduction of tomato. Nickel amendments at 200 or 400 mg/L caused browning and/or necrosis of foliage. Either Ni or nematodes were able to decrease root and shoot growth, and carotenoid and chlorophyll contents of foliage. Nematode disease severity (i.e., degree of galling) increased when plants received a Ni amendment of 50 or 100 mg/L. Nematode reproduction (egg masses/root system) and size of soil population increased when soils were amended with 50 mg Ni/L. Higher concentrations of Ni amendments (200 and 400 mg/L) decreased number of galls, egg masses, fecundity and the soil population of *M. incognita*. Nickel contents of roots, stems and foliage were greater in nematode infected plants than uninfected plants grown in soils receiving no Ni amendments. The order of Ni accumulation was: root > leaves > stem, and leaves > root > stem in infected and uninfected plants, respectively. A small increase in root Ni concentration (up to 24 µg Ni/g dry root) was associated with increased juvenile penetration and gall formation of nematodes; however, further increases of Ni in roots (due to 200 and 400 mg Ni treatments) suppressed nematode pathogenesis. This interaction between *M. incognita* and Ni on dry matter of plant organs was found to be concentration dependent, with the interaction being synergistic at 50 mg/L, but at 400 mg Ni the effect was antagonistic with regard to the effect of Ni on nematodes and plant growth.

Key words: Heavy metal, Leaf pigments, *Lycopersicon esculentum*, *Meloidogyne incognita*, Nickel accumulation.

RESUMEN

Khan, M. R., S. M. Khan, F. A. Mohiddin and T. H. Askary. 2006. Efectos de suelos con alto contenido de níquel sobre el nematodo del nudo radical en tomate. Nematropica 36:79-87.

Los suelos con alto contenido de níquel (Ni) son cada vez más comunes en lugares en donde hay exposición a productos de desecho, y pueden ser nocivos para la agricultura. En un estudio de plantas de tomate (*Lycopersicon esculentum* cv. Pusa Ruby) expuestas a nematodos y alto contenido de níquel en el suelo, se encontró que ambos factores interactúan para afectar el crecimiento de las plántulas. Suelos con 0, 10, 50, 100, 200 y 400 mg/L de níquel y 2000 juveniles de *Meloidogyne incognita/*maceta resultaron en reducción sustancial del crecimiento de las plantas de tomate. Contenidos de níquel de 200 ó 400 mg/L causaron pardeamiento y/o necrosis del follaje. Tanto el Ni como los nematodos redujeron el crecimiento de raíces y parte aérea de la planta, y los niveles de carotenoides y clorofila del follaje. La severidad de la enfermedad causada por los nematodos (grado de agallamiento) aumentó cuando las plantas recibieron 50 ó 100 mg/L de Ni. La reproducción de los nematodos (masas de huevos/sistema radical) y la población en el suelo aumentaron con 50 mg de Ni/L. Las concentraciones más altas de Ni (200 y 400 mg/L) redujeron la cantidad de agallas, masas de hue-

vos, fecundidad y las poblaciones en el suelo de *M. incognita*. El contenido de Ni en las raíces, tallos y follaje fue más alto en las plantas infectadas con nematodos que en plantas no infectadas sembradas en suelos sin adición de Ni. El orden de acumulación de Ni en plantas infectadas fue raíces > hojas > tallos, y en plantas no infectadas fue hojas > raíces > tallos. Pequeños aumentos en la concentración de Ni (hasta 24 µg Ni/ g de raíces secas) estuvieron asociados con aumento en la penetración de juveniles y formación de agallas. Sin embargo, aumentos por encima de esta cantidad de Ni en las raíces (debido a los tratamientos de 200 y 400 mg de Ni/L) suprimieron la patogénesis del nematodo. Se encontró que esta interacción entre *M. incognita* y Ni en la materia seca de los órganos de la planta es dependiente de la concentración. La interacción fue sinergística a 50 mg/L, pero antagonística a 400 mg/L con repecto al efecto del Ni en los nematodos y el crecimiento de la planta.

Palabras clave: Metal pesado, pigmentos foliares, *Lycopersicon esculentum*, *Meloidogyne incognita*, acumulación de níquel.

INTRODUCTION

Nickel (Ni) is a natural component of soils and is also an essential plant nutrient (Brown *et al.*, 1987; Wood *et al.*, 2004; Bai *et al.*, 2006). However, at high soil concentrations, Ni can be toxic to many plant species. Nickel contamination can occur if wastes or municipal and industrial sewage is applied to soils, especially if Ni is in the form of mobile organic chelates (Kabata-Pendias and Pendias, 1999). The metal processing industry also contributes to localized soil accumulation of Ni, causing soil Ni levels as high as roughly 1500 mg/kg (Hutchinson and Whitby, 1974). Soil Ni levels at 20 mg/kg can cause crop toxicity and reduce growth and yield (Heale and Ormrod, 1982; Bisessar *et al.*, 1983; Khan *et al.*, 1996). Nickel toxicity symptoms often include marginal and interveinal chlorosis, decreased leaf pigmentation, smaller leaves, premature senescence, and smaller roots that are also discolored (Hale *et al.*, 1985; Khan *et al.*, 1987).

The population characteristics of soilborne plant pathogens are substantially influenced by the physical and chemical characteristics of soils. Therefore, excessive soil Ni may potentially influence population and pathogenesis of plant pests, such as parasitic nematodes. Bisessar *et al.*

(1983) reported increased severity of root-knot disease caused by *Meloidogyne hapla* on celery grown in soil contaminated with Ni at 7500 mg/kg, Cu at 800 mg/kg, and Co at 100 mg/kg soil. Others have also demonstrated that Ni, and other heavy metals, can influence parasitism by nematodes (Olthof and Potter, 1972; Temple and Bisessar, 1981; Khan *et al.*, 1996). These researchers indicate that metal contamination of soils may influence pathogenesis of root-knot nematodes and that nematode infection can influence metal accumulation in plant parts (Nyczepir *et al.*, 2006). To ascertain root-knot nematode and soil nickel interactions, a pot trial was undertaken to examine the effects of Ni amendment (10, 50, 100, 200 and 400 mg Ni/L) on root penetration, disease severity (galls/root system), reproduction (egg masses/root system, eggs/egg mass), soil population of *M. incognita,* plant growth, yield, leaf pigments and Ni concentration of tomato plants.

MATERIALS AND METHODS

Treatments

The study was conducted using tomato plants growing in a soil mix comprised of 1.5 kg autoclaved soil (loam and compost

3:1; pH, 6.2; CEC, 4.1; C/N ratio, 9.3) in 15 cm diam. clay pots. Treatments consisted of five concentrations of Ni as ($NiCl_2$ at 0, 10, 50, 100, 200 and 400 mg/L) using distilled water (to 18 ohms).The initial Ni concentration of the "0-Ni" soil treatment was 6.2 mg/kg soil. Individual pots received 480 ml of the relevant Ni solution so as to achieve homogenous distribution of Ni in the potted soil. Pots were then left unplanted for one week to achieve equilibrium between added Ni and soil organic matter.

Plant Culture

Three week old tomato seedlings (*Lycopersicon esculentum* Mill cv. Pusa Ruby; raised in autoclaved soil) were transplanted to pots (1 seedling/pot) the last week of October. One week later, in one set of pots, each seedling was inoculated with 2000 freshly hatched juveniles of *M. incognita* (Kofoid and White) Chitwood. A set of pots without Ni solution served as a control. All the treatments were replicated five times using one pot per experimental unit. The pots were arranged in a randomized complete block design in an open field exposed to natural light conditions. Mean temperature, relative humidity and rainfall during November-February were 19.0°C (13-25.4°C) and 61% (48-79%) and 31.3 mm (0.0-4.8 mm), respectively. The plants were irrigated with tap water having 3.8 mg Ni/L on alternate days. Treatment plants were observed during the growth period for appearance of symptoms of Ni toxicity or nematode infection. The plants were harvested in February, three and a half months after inoculation, and dry weight of shoots and roots, leaf pigments, Ni accumulation, and nematode disease were determined.

Nickel and Photosynthetic Pigment Contents

Plants were dried in a hot-air oven at 45°C for 72 h and their leaves, stems, and roots were ground separately to a coarse powder. A one gram sample from plant organs of each treatment (root, leaves, stem) was digested with 10 ml of acid [HNO3:HClO4 (4:1)]. The digested sample was heated on a hot plate until cessation of release of brown fumes and the digested mass converted into a liquid releasing white fumes (Piper, 1966). The acid residue was then dissolved in 5 ml concentrated HCl and diluted with 5 ml distilled water. The solution was filtered through Whatman filter paper no. 1. The filtrate was collected in a volumetric flask and further diluted to 50 ml with distilled water. The concentration of Ni in roots, stems and leaves was estimated using atomic spectrophotometry (Horwitz, 1970).

Chlorophylls and carotenoids were extracted by macerating fresh leaf tissue in 85% acetone (Lichtenthaler, 1987). One gram fresh leaf tissue pooled from 5 plants (replicates) of a treatment was placed in a mortar and pestle on ice and half-filled with liquid nitrogen. A small amount of grinding sand was also added. After evaporation of the liquid nitrogen, leaf tissue was ground by pestle to a course powder. One ml of 85% acetone (pH 8) was then added and ground further. The suspension was poured into a 1.5 ml tube and centrifuged at 10,000 rpm for 3 minutes. One ml of supernatant was diluted to 3 ml with 85% acetone and absorbance read in a spectrophotometer at 637 nm (for carotenoid), 647 and 663 nm (for chlorophylls). If absorbance was greater than 1.0, the solution was further diluted with acetone.

Root Penetration, Disease Severity and Nematode Reproduction

The effect of Ni on root penetration by *M. incognita* larvae was assessed on three-week- old seedlings of tomato cv. Pusa Ruby planted in plastic cups containing 100 g

autoclaved soil (same as used above). Treatments consisted of Ni solutions of different concentrations added to each cup just before planting (35 ml/cup). A 5 ml nematode suspension, containing 500 freshly hatched juveniles of *M. incognita,* was added two days later to each cup. Seedlings were uprooted and root penetration was determined two weeks later (Southey, 1986).

Root galls and egg masses were counted from freshly harvested plants grown three and a half months in clay pots. The entire root system was examined under a stereomicroscope to count galls and egg masses. Egg masses were stained by holding roots in phloxin B solution (0.95 g/L water) for 20 minutes so as to facilitate counting. Fecundity (number of eggs/egg mass) was determined from 100 egg masses excised from five roots (replicates) of each treatment. Egg masses were shaken in a 1% NaOCl solution in an electric blender to liberate eggs. Eggs were subsequently counted under a stereomicroscope (Khan and Khan, 1994). Soil population of *M. incognita* was estimated by Cobb's sieving and decanting method followed by Baermann's funnel technique (Southey, 1986).

Statistical Analysis

The study was performed over two consecutive years, repeating all experiments in the second year. Treatment effects were generally consistent between years, yet year effects appeared to exist; thus, data obtained from the five replicates maintained each year were analyzed separately. Data reported here describe second year results, although first year results were very similar. The data on plant growth, leaf pigments and Ni accumulation were subjected to a two-way single-factor analysis of variance (ANOVA) with least significance differences (LSD) calculated at P = 0.05 (Dospekhov, 1984). Root penetration, gall

formation, egg mass production, fecundity and soil populations were analyzed by the one-way single-factor ANOVA and LSD were calculated at P = 0.05. Regression analysis for certain variables was performed for curvilinear or linear relationships. Standard deviation (SD) was calculated for each treatment and has been indicated on the regression lines.

RESULTS

Symptoms

Nickel treated soils (except those treated with 10 and 50 mg/L) produced plants with stunted growth and sparse branching. Nickel at 200 or 400 mg/L induced interveinal chlorosis and necrosis of leaves, with fewer and smaller leaves that also senesced prematurely. Leaves also exhibited curling, especially at 400 mg Ni/L. The 50 or 100 mg Ni treatments caused leaves to be chlorotic. Root lateral growth diminished at 200 and 400 mg Ni/L. Root-knot nematode, at an inoculum level of 2000 J2/plant, caused extensive galling of roots. The nematode did not cause any discernible effect on Ni toxicity symptoms, but Ni treatments influenced nematode disease severity. Treatments involving Ni at 10 or 50 mg/L produced severe gall formation, whereas, at 400 mg Ni, infection was moderate.

Plant Growth

Nematode infection decreased ($P \leq$ 0.05) the dry weight of shoots and roots of tomato plants compared to uninoculated control plants (Fig. 1). Treatments with nickel also reduced the dry weight of inoculated (except 10 mg Ni/L) and uninoculated plants (except 10 and 50 mg Ni/L) compared to their respective controls. The two-way ANOVA revealed that the reduction in the dry matter of plants due to Ni
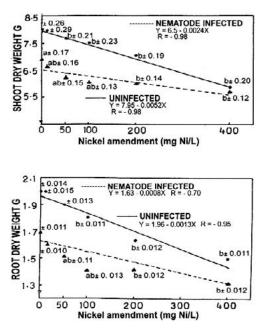
Fig. 1. Effect of Ni and root-knot nematode, *Meloidogyne incognita*, on dry matter production of tomato. a Significantly different from corresponding uninoculated treatment at the same concentration of Ni at *P* = 0.05; b Significantly different from 00 mg Ni/L at *P* = 0.05; ab Significantly different from corresponding uninoculated treatment at the same concentration of Ni as well as from 00 mg Ni/L at *P* = 0.05; ± Standard Deviation.

application was greater in nematode treatments than without nematodes. The differences of dry weights of inoculated and uninoculated treatments at the Ni concentrations used were significant (*P* ≤ 0.05); *F*-values (df = 1) were significant at 50 and 100 mg Ni/L for root dry weight and at 50 mg Ni/L for shoot dry weight and root length at *P* ≤ 0.05. Combined effect of nematodes and Ni varied considerably. At lower concentration, i.e. 10 and 50 mg Ni/L, reduction in plant dry weight was greater than the sum of the reductions caused by the nematodes and Ni separately. At the other concentrations, the joint effects were nearly additive (100 and 200 mg Ni/L) or antagonistic (400 mg Ni/L).

Leaf Pigments

Nematode infection and Ni treatments (except 10 mg Ni/L) caused significant reduction in chlorophyll-a and total chlorophyll of tomato leaves (Fig. 2). Chlorophyll-b decreased (*P* ≤ 0.05) as Ni increased from 100, 200 and 400 mg Ni/L in inoculated plants and at 200 and 400 mg Ni/L in uninoculated plants (Fig. 2). Carotenoid content of leaves was significantly lower for Ni treatments for both inoculated (100, 200 and 400 mg Ni/L) and uninoculated nematode treatments (200 and 400 mg Ni/L) compared to the respective controls (Fig. 3). The two-way ANOVA revealed significant differences between inoculated and uninoculated treatments for chlorophyll-a, and for chlorophyll-b at 400 mg Ni/L, and for total chlorophyll at 50 and 100 mg Ni/L and for carotenoids at 100 mg Ni/L. *F*-values of Ni treatments (df = 5) were significant (*P* ≤
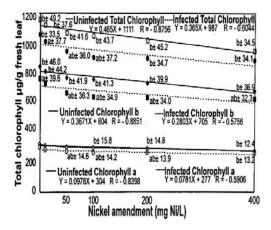

Fig. 2. Effect of Ni and root-knot nematode, *Meloidogyne incognita*, on chlorophyll-a, chlorophyll-b and total chlorophyll content of tomato leaves. a Significantly different from corresponding uninoculated treatment at the same concentration of Ni at *P* = 0.05; b Significantly different from 00 mg Ni/L at *P* = 0.05; ab Significantly different from corresponding uninoculated treatment at the same concentration of Ni as well as from 00 mg Ni/L at *P* = 0.05; ± Standard Deviation.
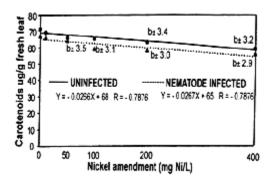
Fig. 3. Effect of Ni and root-knot nematode, *Meloidog-yne incognita*, on carotenoid content of tomato leaves. a Significantly different from corresponding uninoculated treatment at the same concentration of Ni at P = 0.05; b Significantly different from 00 mg Ni/L at P = 0.05; ab Significantly different from corresponding uninoculated treatment at the same concentration of Ni as well as from 00 mg Ni/L at P = 0.05; ± Standard Deviation.

0.05) for chlorophyll pigments of both inoculated and uninoculated plants.

Nickel Accumulation in Plant Parts

Nickel concentration in roots, stems and leaves of tomato plants increased by 32-2097% with the increase in soil Ni concentration from 10 mg to 400 mg Ni/L (Fig. 4). The increase was significant at all concentrations except 10 mg Ni/L in uninoculated plants. The two-way ANOVA revealed that Ni concentrations of inoculated plants were significantly greater than those of the uninoculated plants. The greatest increase in the metal contents of inoculated plants compared to uninoculated plants occurred at 10 mg Ni. Root nickel was increased by 44% with 10 mg Ni/L, followed by 40% with 50 mg Ni/L. *F*-values for both these treatments (df = 1) were significant at $P \leq 0.05$. Nickel contents of stems and leaves of infected plants compared to uninfected plants were increased by 76 and 55%, respectively at 10 mg Ni/L, *F*-values were significant at this concentration only. The difference in Ni
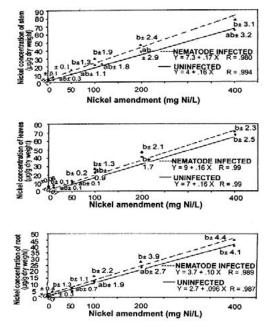

Fig. 4. Ni accumulation in plant parts in the presence and absence of root-knot nematode, *Meloidogyne incognita*.a Significantly different from corresponding uninoculated treatment at the same concentration of Ni at P = 0.05; b Significantly different from 00 mg Ni/L at P = 0.05; ab Significantly different from corresponding uninoculated treatment at the same concentration of Ni as well as from 00 mg Ni/L at P = 0.05; ± Standard Deviation.

contents of inoculated and uninoculated plants, however, decreased gradually with the increase in the metal concentration, and was lowest, i.e., 16% (root), 10% (stem) and 4% (leaves), at 400 mg Ni. Nickel concentrations of inoculated and uninoculated plants generally followed a pattern of roots > leaves > stems and leaves > stems > roots, respectively.

Nematode Disease

Root penetration by juveniles of *M. incognita* in tomato seedlings and gall formation (number of galls/root system) increased ($P \leq 0.05$) due to increasing soil Ni concentrations, with 50 or 100 mg/L

being greatest compared to the control (Fig. 5). At 400 mg Ni, the penetration and galling decreased significantly. Egg mass production (number of egg masses/root system) significantly increased at 50 mg/L (22%) and decreased at 200 (26%) and 400 mg Ni (45%) compared to the control. The fecundity (number of eggs/egg mass) was decreased ($P \leq 0.05$) at 200 and 400 mg Ni (Fig. 5). Soil population of *M. incognita* increased significantly at 50 mg Ni (13%), but decreased at 200 (17%) and 400 mg (51%) compared to the control (Fig. 5). The ANOVA revealed significant *F*-values at $P \leq 0.05$ for all variables.

DISCUSSION

Elevation of soil Ni proved to be potentially toxic to tomato plants, causing chlo-
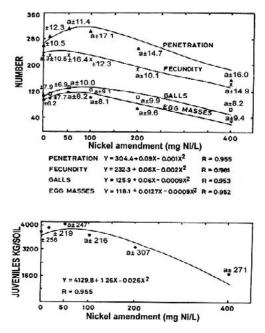

Fig. 5. Effect of Ni on root penetration, gall formation, egg mass production, fecundity and soil population of root-knot nematode, *Meloidogyne incognita.* a Significantly different from 00 mg Ni/L at *P* = 0.05; ± Standard Deviation.

rosis and necrosis of leaves and diminished growth. Similar symptoms of Ni toxicity have been reported on lettuce (Temple and Bisessar, 1981), celery (Bisessar *et al.*, 1983) and tomato (Hale *et al.*, 1985). Nickel accumulated in organs of both nematode infected and uninfected plants, but accumulation was greatest in the former. In nematode infected plants, Ni accumulation was greater in roots than leaves or stems, or any part of uninoculated plants. The cause of this nematode induced accumulation is speculative, but nematode infection can potentially disrupt translocation of nutrients from roots to aerial organs, and thus causes their accumulation in roots (Wilcox-Lee and Loria, 1987). Perhaps this is the reason why Ni concentration in roots of infected plants was greater than in roots of noninfected plants. These data indicate the possibility that, due to disruption of root physiology, Ni translocation to shoots of infected plants is less controlled than in noninfected plants; thus, increasing Ni accumulated in aerial organs of infected plants. A similar accumulation of Ni in nematode infected plants was found by Bisessar *et al.* (1983) for celery plants infected with *M. hapla.* In contrast, Nyczepir *et al.*, (2006) observed the association of *M. partityla* in the Ni deficiency in pecan seedlings grown in soil with normal levels of Ni. Thus root-knot nematodes may not always increase Ni contents. This indicates that Ni accumulation in plants due to root-knot nematode infection may vary with the initial Ni concentration in the soil.

Accumulated Ni caused toxicity on tomato leaves, which appeared in the form of yellowing, chlorosis or necrosis. Leaf pigments are highly sensitive to excess Ni. Excess Ni inhibits chlorophyll biosynthesis and induces degradation (Krupa *et al.*, 1993; Abdel-Basset *et al.*, 1995) with a subsequent decrease in chlorophyll concen-

tration (Molas, 2002) that is manifested as chlorosis and/or necrosis of foliage.

Certain soil Ni treatments (10-50 mg/L) facilitated pathogenesis of *M. incognita,* as evidenced by increased root penetration by larvae. Root penetration is key to pathogenesis, and subsequently leads to gall formation (Bird, 1968). Especially high soil Ni, at 200 or 400 mg/L, appeared to be toxic to larvae, thus root penetration and gall formation decreased. This observation contrasts another study where 7500 mg Ni/kg soil significantly increased galls on celery roots (Bisessar *et al.*, 1983). That study was conducted in ambient conditions with soil contaminated with other metals (i.e., 800 mg Cu and 100 mg Co/kg soil), which may have influenced the response of celery plants. Nematode reproduction therefore appears to be potentially sensitive to soil Ni concentrations.

The 50 mg/L soil Ni treatment promoted pathogenesis of *M. incognita*, suggesting a synergistic interaction between the nematode and Ni (50 mg) that led to a greater decrease in plant growth variables compared to the sum of decrease caused by *M. incognita* and 50 mg Ni/L individually. For example, 50 mg Ni and the nematode separately decreased the shoot dry weight of tomato by 3 and 14%, respectively, but jointly they caused a 24% reduction. Apparently, this occurred due to increase in nematode disease severity and greater accumulation of the metal in infected plants grown in 50 mg Ni/L treated soil. Higher soil Ni concentrations, especially 400 mg Ni greatly suppressed nematode penetration (32%) and galling (45%), leading to relatively little damage to the root system by nematodes. This resulted in an antagonistic interaction, where a decrease in plant growth parameters was less than the sum of individual effects. For example, the sum of the reductions in shoot dry weight of tomato caused

by the nematodes and 400 mg Ni separately was 40% compared to the joint effect of 28%. At 100 or 200 mg Ni/L, the interactive effects with the nematode were additive.

This study demonstrates a concentration-dependent relationship of soil Ni with root-knot nematodes. Nematode infection can increase plant sensitivity to high Ni soils, and soil Ni at certain relatively high concentrations can enhance nematode infection, leading to substantial decreases in plant productivity. Certain soil Ni levels (50 and 100 mg Ni/L) can contribute to elevated soil nematode populations. High soil Ni concentration such as 400 mg Ni/L can potentially be nematicidal. It is also concluded that soils contaminated with waste containing Ni can potentially affect crop yields directly via Ni toxicity and indirectly via enhancing nematode damage.

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