Ability of nitrogen containing salts to control the root-knot nematode (*Meloidogyne javanica*) on tomato

M.R. Karajeh* and F.M. Al-Nasir

**Summary**  The influence of different nitrogen containing salts and sodium chloride at gradual electrical conductivity levels (ECs 2, 4, 6 and 8 mS/cm) on the root-knot nematode (*Meloidogyne javanica*) and their interaction with tomato was evaluated under growth chamber and greenhouse conditions. Both ammonium chloride (NH$_4$Cl) and ammonium sulfate ((NH$_4$)$_2$SO$_4$) were more effective than ammonium nitrate (NH$_4$NO$_3$) which was more effective than potassium nitrate (KNO$_3$) and sodium chloride (NaCl) in suppressing *M. javanica* by reducing root galling and nematode reproduction on tomato cv. GS12. Under greenhouse conditions, the minimum significant galling index values assessed for NH$_4$Cl, (NH$_4$)$_2$SO$_4$, NH$_4$NO$_3$ and KNO$_3$ were 1.60, 2.04, 2.30 and 3.30, respectively whereas the maximum value (4.01) corresponded to NaCl and was not statistically different from the control (4.92). A significant increase in tomato growth and protein content for (NH$_4$)$_2$SO$_4$ and NH$_4$NO$_3$ was observed. On the other hand, in NaCl treatment, there was a decrease in dry weights and protein content due to salinity compared with the control. The higher salt ECs did not affect the pH of the rhizospheric soil but slightly increased its measured EC and salinity. Hence, (NH$_4$)$_2$SO$_4$ is a more suitable candidate than NH$_4$Cl for the effective control of the root-knot nematode when irrigation water is a limiting factor with high salinity level similar to NaCl. Therefore, the use of ammonium containing salts especially (NH$_4$)$_2$SO$_4$ and NH$_4$Cl alone or in combination with other control measures may result in controlling *M. javanica*.

**Additional keywords:** fertilizers, management, *Solanum lycopersicum*, suppression

**Introduction**

Root-knot nematodes (*Meloidogyne* Goldi 1892 - RKN) are obligate parasites of higher plants distributed worldwide and considered major nematode pests, causing great crop losses annually (Sasser, 1987; Sasser and Freckman, 1987; Nickle, 1991) and reduction of product quality on almost every plant species (Anastasiadis et al., 2011).

Tenuta and Ferris (2004) reported that *Meloidogyne javanica* (Treub) Chitw. reared on solid medium and in hydroponic culture, were slightly more sensitive to specific ion and osmotic effects than nematodes of similar colonizer-persister groups obtained from soil. Also, gradients of salts of the specific ion repellents (NH$_4^+$, K$^+$, Cl$^-$, and NO$_3^-$) for *M. incognita* have been demonstrated to shield tomato (*Solanum lycopersicum* Mill.) roots from root knot nematode infection in soil (Marks and Sayre, 1964; Edongali and Ferris, 1982; Ismail and Saxena, 1977; Castro et al., 1991), furthermore, NH$_4^+$, K$^+$ and NO$_3^-$ have been recognized as beneficial for plant growth (Castro et al., 1991). Root galling and *M. incognita* reproduction efficiency increased in tomato plants exposed to ammonia at 76μg/m$^3$, while treatments with higher concentration (152μg/m$^3$) caused nematode suppression (Khan and Khan, 1995). Additionally, urea and ammonium sulfate at rates of more than 250mg N/kg soil resulted in suppression of *M. incognita* population on tomato plants (D’Addabbo et al., 1996).

Stimulation or depression effects of some salts on various plant physiological functions are already known, especially when combined with other stressful agents such as nematodes (Edongali and Ferris, 1982).
The objectives of this study were to investigate the effects of five salts at four electrical conductivity (EC) levels (2, 4, 6 and 8 mS/cm) on the control of the root nematode *M. javanica*. The test salts comprised of four nitrogen containing salts; ammonium chloride (NH₄Cl), ammonium nitrate (NH₄NO₃), potassium nitrate (KNO₃) and ammonium sulfate ((NH₄)₂SO₄), and sodium chloride (NaCl). The interaction of the effects of the salts, the EC levels and the root-knot nematode on the susceptibility and growth of tomato were also assessed under growth chamber and greenhouse conditions.

**Materials and Methods**

Analytical reagent grade of five salts, NH₄Cl, NH₄NO₃, KNO₃, NaCl and (NH₄)₂SO₄, was used. Electrical conductivity for each salt solution used was 2 (EC2), 4 (EC4), 6 (EC6) and 8 (EC8) mS/cm and was achieved by dissolving 1.07, 2.14, 3.21 and 4.28 g/l NH₄Cl, 1.60, 3.20, 4.80 and 6.39 g/l NH₄NO₃, 2.02, 4.04, 6.06 and 8.08 g/l KNO₃, 1.17, 2.34, 3.50 and 4.67 g/l NaCl or 2.63, 5.28, 7.92 and 10.55 g/l (NH₄)₂SO₄, respectively, in water.

A field population of *M. javanica* previously collected from a cucumber field at Ein-Sarah region in Karak Province of Jordan and extracted in the laboratory was used for this study. The nematode population was morphologically identified and molecularly characterized using the sequence characterized amplified region-polymerase chain reaction (SCAR-PCR) test (Karajeh, 2004).

**Infectivity test**

Two-week-old seedlings (ca. 5 cm tall) of *M. javanica*-susceptible tomato cv. GS12 were transplanted into 100 ml plastic pots (one seedling/pot), filled with a sterilized mixture of 1:1:1 peat: sand: perlite. For each treatment, one thousand 2nd stage juveniles (J2s) of *M. javanica* were picked and transferred into a small Petri dish containing 10 ml of each solution at each EC or sterile tap water (served as control). The juveniles were two-days-old and hatched from eggs which were surface-sterilized with 0.5% NaOCl.

All Petri dishes were kept at room temperature for one hour before inoculating the tomato seedlings through pouring the solutions into the rhizospheric region of each seedling one week after transplanting. Each treatment was replicated five times. The plants were regularly irrigated with water. The treated and control plants were arranged according to a completely randomized design (CRD) and maintained in a growth chamber at 25°C and 16/8 hour light/dark regime.

Six weeks after inoculation, the plants were up-rooted and the galling index evaluated according to the 0-5 scale: 0=no galling, 1=1-2 galls, 2=3-10, 3=11-30, 4=31-100 and 5=over 100 galls (Taylor and Sass- er, 1978). Egg-masses were picked from the roots, extracted with a 0.5% NaOCl solution for 30 seconds (Hussey and Barker, 1973) and quantified under a compound microscope at 10X magnification level. Nematode reproduction factor (RF) was calculated as the number of eggs per plant (Pf) divided by the initial J2 inoculum number (Pi).

**Greenhouse experiment**

To determine the effects and interactions of *M. javanica* with variable levels of electrical conductivity of the five salts on the growth of tomato in greenhouse conditions, one-month old seedlings (about 15cm tall) of tomato cv. GS12 were tested. The seedlings were planted in 5dm³ pots filled with 1.5 kg of a 1:2 mixture of water-washed sand and a non-sodic, non-saline sandy loam soil (EC 1.3 mS/cm, pH 7.1, 0.6% organic matter, 1.1mg/g total nitrogen, and 43% CaCO₃) that had been previously sterilized at 85°C for 5 days. One week after transplanting, the desired level of electrical conductivity (EC2, EC4 and EC8) was achieved and maintained by irrigating the soil with saline solutions to field capacity level for three consecutive weeks. For the control treatments, the same procedure was followed except that tap water replaced the salt solution.

Each treatment was replicated six times (one plant per pot) and each seedling was
Control of *Meloidogyne javanica* with N-containing salts on tomato

Inoculation was performed one day after salt treatment by pouring the egg suspension of the nematode into three holes made in the rhizospheric soil. Non-inoculated plants were used as controls. The plants were transferred into a greenhouse (25 ± 5 °C air temperature and 12/12 hour light/dark regime) and maintained without fertilization. All treatments were arranged in a randomized complete block design (RCBD).

At the end of the experiment, sixty days post-inoculation, plants were removed from the pots and the roots were carefully washed to remove soil particles. Fresh and dry weights of plant shoots and roots were recorded. The galling index and nematode reproduction factor were evaluated as previously described. After sieving and grinding of 10 g of the rhizospheric soil (oven dried) and dissolving it in distilled water, soil pH, EC and salinity were measured using PCSTestr35 (Eutech Instruments, USA). Representative random samples of oven dried plants were finely ground and analyzed for protein, potassium and phosphorus content (Lowry *et al.*, 1951; Meiwes *et al.*, 1984).

### Statistical analysis

Data were analyzed statistically using general linear model (GLM) procedure (SPSS software version 11.5; SPSS Inc., Chicago, USA). Significance of main factors and interactions was tested at the 0.05 probability level. Least significance difference (LSD) test was used for mean separation at the 0.05 probability level.

### Results

Under controlled growth conditions, all nitrogen containing salts were able to reduce the extent of root galling over the control, with the exception of NaCl with EC levels at 2, 4 and 6 mS/cm. Both NH₄Cl and (NH₄)₂SO₄ were relatively more effective in reducing root galling index to less than 2 as compared to KNO₃ and NH₄NO₃ (Figure 1a) and this reduction was accompanied by a significant reduction in nematode reproduction expressed as lower RF values (Figure 1b).

Results from the greenhouse experiment revealed that nitrogen containing salts caused significant reduction in tomato root galling index, compared to the NaCl treatment and control, and this reduction was clearer at higher EC levels (Table 1). The minimum significant galling index value was observed for the NH₄Cl treatment (1.60), regardless the EC level, followed by the (NH₄)₂SO₄ (2.04), NH₄NO₃ (2.30), KNO₃ (3.30), NaCl treatments (4.01) and the control (4.92) (Table 1). A similar pattern was observed in the case of RF values since KNO₃, NaCl and control caused profound nematode reproduction, while the other salts showed a significantly reduced reproduction (Table 1).

There was a significant increase in tomato plant and root dry weights and protein content in the treatments (NH₄)₂SO₄ and NH₄NO₃ over the control whereas the treatments NH₄Cl and KNO₃ were not significantly different from the control. Nevertheless, a decrease in dry weight and protein content was observed in the NaCl treatment compared to the control (Table 1). In general, there was a significant reduction in phosphorus plant content in the salt treatments over the control. The highest and most significant potassium content was observed for the KNO₃ treatment (ca. 40 g/kg) compared to the other salts and the control. There were no significant pH differences among the treatments. As the EC level increased, there was a gradual increase of soil EC. The highest measured EC was recorded for the NaCl treatment (3.33 mS/cm) at the highest EC (EC 8) and the lowest for the control (1.84 mS/cm). Measured salinity was significantly higher for NaCl and NH₄Cl treatments than for the other treatments. Potassium nitrate did not cause a significant increase in the level of measured salinity over the control (Table 1).

Root galling of tomato plants was affected mainly by the nematode, salt type and the interaction between them and by their interaction with EC (Table 2). The salt effect was significant on plant and root fresh
and dry weights and on the measured EC ($P \leq 0.05$). The presence or absence of nematode was important for root dry weight. Electrical conductivity level, as a main factor, had no significance on the tested parameters (Table 2).

**Discussion**
Tomato is a high fertilizer-input crop in which the form of nitrogen is particularly important because it influences plant growth (Atherton et al., 1986) and plant response to a range of diseases (Hendrix and Toussoun, 1964; Huber and Watson, 1974). Ammonium chloride was previously reported to be more effective than KNO$_3$ on tomato (Karajeh and Al-Nasir, 2008) and this result was confirmed in this study where another two ammonium-containing salts ($\text{(NH}_4\text{)}_2\text{SO}_4$ and $\text{NH}_4\text{NO}_3$) were additionally tested. Ammonium sulfate was as effective as $\text{NH}_4\text{Cl}$ but $\text{NH}_4\text{NO}_3$ was less effective than both of these salts and more effective than KNO$_3$ and NaCl in suppressing *M. javanica* by reducing the rate of root galling and nematode reproduction on tomato under laboratory and greenhouse conditions. Akhtar and Mahmood (1994) reported that the addition of ($\text{NH}_4\text{)}_2\text{SO}_4$ (110kg N/ha) reduced the total population of plant-parasitic nematodes as well as root-galling induction by *M. incognita* on tomato, whereas it increased the number of free-living nematodes. Other ammonia-releasing compounds; $\text{NH}_4\text{OH}$, ($\text{NH}_4\text{)}_2\text{HPO}_4$ and $\text{NH}_4\text{HCO}_3$, were also found effective and showed the greatest nematicidal activity on *M. javanica* at concentrations of 300mgN/kg soil. In a field experiment, the nematicidal efficacy of $\text{NH}_4\text{OH}$ on tomato plants at doses of 1000 and 2000kgN/ha was equivalent to those of metham sodium in combination with cadusafos (Oka and Pivonia, 2002). Furthermore, the application of ammonium salts had a nematicidal effect against *Pratylenchus penetrans* (Cobb) Filipjev et Schuurmans Stekhoven (Walker,
Table 1. Effect of four nitrogen containing salts and NaCl at four levels of electrical conductivity (EC) on the root galling index and reproduction factor of the root-knot nematode, *Meloidogyne javanica*, in tomato pot plants and on plant and soil properties.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Type</th>
<th>EC&lt;sup&gt;1&lt;/sup&gt; (mS/cm)</th>
<th>GI (0-5)</th>
<th>RF</th>
<th>PDW (g)</th>
<th>RDW (g)</th>
<th>P Content (g/kg)</th>
<th>K Content (g/kg)</th>
<th>Protein content (mg/g)</th>
<th>pH (0-14)</th>
<th>EC (mS/cm)</th>
<th>Salinity (ppt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;Cl</td>
<td>2</td>
<td>2.31&lt;sup&gt;1&lt;/sup&gt; d&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.21 d</td>
<td>9.14 c</td>
<td>2.19 bc</td>
<td>1.56 d</td>
<td>34.31 c</td>
<td>165.23 a</td>
<td>6.58 a</td>
<td>2.11 d</td>
<td>1.04 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.68 d</td>
<td>1.35 d</td>
<td>10.29 b</td>
<td>1.92 bc</td>
<td>1.52 d</td>
<td>34.52 c</td>
<td>56.10 d</td>
<td>6.70 a</td>
<td>2.92 b</td>
<td>1.50 ab</td>
<td></td>
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<tr>
<td></td>
<td>8</td>
<td>1.60 d</td>
<td>1.31 d</td>
<td>8.29 cd</td>
<td>1.90 bc</td>
<td>1.99 b</td>
<td>37.50 b</td>
<td>91.33 c</td>
<td>6.42 a</td>
<td>3.14 a</td>
<td>1.65 a</td>
<td></td>
</tr>
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<td>(NH&lt;sub&gt;4&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>2</td>
<td>2.89 cd</td>
<td>2.31 d</td>
<td>11.40 b</td>
<td>2.66 ab</td>
<td>1.86 bc</td>
<td>31.00 d</td>
<td>108.51 bc</td>
<td>6.60 a</td>
<td>1.88 e</td>
<td>0.94 c</td>
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<td></td>
<td>4</td>
<td>2.33 cd</td>
<td>1.84 d</td>
<td>12.61 ab</td>
<td>2.35 b</td>
<td>1.41 d</td>
<td>34.44 c</td>
<td>135.40 b</td>
<td>6.51 a</td>
<td>2.20 d</td>
<td>1.12 c</td>
<td></td>
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<td>8</td>
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<td>1.67 d</td>
<td>12.59 ab</td>
<td>1.67 bc</td>
<td>1.24 e</td>
<td>31.63 d</td>
<td>150.33 a</td>
<td>6.48 a</td>
<td>2.63 bc</td>
<td>1.30 b</td>
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<tr>
<td>KNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2</td>
<td>3.85 b</td>
<td>8.40 b</td>
<td>10.97 ab</td>
<td>1.50 a</td>
<td>1.75 c</td>
<td>37.51 b</td>
<td>91.13 c</td>
<td>6.55 a</td>
<td>2.54 c</td>
<td>1.22 bc</td>
<td></td>
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<tr>
<td></td>
<td>4</td>
<td>3.30 bc</td>
<td>4.35 c</td>
<td>9.74 c</td>
<td>1.73 bc</td>
<td>1.40 d</td>
<td>40.65 a</td>
<td>55.64 d</td>
<td>6.50 a</td>
<td>2.60 bc</td>
<td>1.28 bc</td>
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<td>3.60 cd</td>
<td>9.18 c</td>
<td>1.74 bc</td>
<td>1.88 bc</td>
<td>40.50 a</td>
<td>92.75 c</td>
<td>6.55 a</td>
<td>2.70 bc</td>
<td>1.20 bc</td>
<td></td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;NO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2</td>
<td>3.44 b</td>
<td>4.69 cd</td>
<td>12.81 ab</td>
<td>2.21 bc</td>
<td>1.88 bc</td>
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<td>3.58 cd</td>
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<td>1.75 c</td>
<td>1.75 c</td>
<td>31.23 d</td>
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<td>2.20 d</td>
<td>1.23 bc</td>
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<tr>
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<td>8</td>
<td>2.70 cd</td>
<td>3.90 c</td>
<td>14.88 a</td>
<td>1.75 c</td>
<td>1.88 bc</td>
<td>31.14 d</td>
<td>109.47 bc</td>
<td>6.55 a</td>
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<td>NaCl</td>
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<td>4.86 a</td>
<td>12.71 a</td>
<td>6.05 e</td>
<td>1.58 cd</td>
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<td>91.89 c</td>
<td>6.64 a</td>
<td>2.75 bc</td>
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<tr>
<td></td>
<td>8</td>
<td>4.01 ab</td>
<td>4.17 c</td>
<td>9.58 c</td>
<td>1.74 bc</td>
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</tr>
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<td>Control</td>
<td>0</td>
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<td>13.34 a</td>
<td>8.66 cd</td>
<td>1.54 cd</td>
<td>2.17 b</td>
<td>31.24 d</td>
<td>106.25 bc</td>
<td>6.55 a</td>
<td>1.84 e</td>
<td>0.91 c</td>
<td></td>
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</tbody>
</table>


<sup>2</sup> Average of 6 replicates per each treatment.

<sup>3</sup> Means within columns followed by the same letters are not significantly different at 0.05 probability level using LSD.
A higher ammonium level in nutrient medium, in the presence or absence of excised roots, decreased the total number of J2s that hatched from dispersed eggs and egg-masses (Sudirman and Webster, 1995). Increasing of ammonium levels reduced the percentage of J2s, which penetrated the roots over time as compared to the control (Sudirman, 1992).

The mode of action of ammonium on the root-knot nematode can be explained by the assumption that higher concentrations of ammonium may have been sufficient to modify malate dehydrogenase activity (Viglierchio, 1979). This would have subsequently a) decreased the energy available for egg hatching and plant invasion processes and/or b) significantly affected the electrical potential around the root tip area where J2s penetrated and thus influenced nematode penetration of the roots through diminished attractiveness of the root tips (Scott and Martin, 1962) and/or c) significantly inhibited giant cell formation and nematode development without affecting root growth (Orion et al., 1980; Orion et al., 1995).

Increasing the salt EC level from EC2 up to EC8 resulted in decreasing the development of the root-knot nematode on tomato and increasing plant growth. The higher salt EC did not affect the pH of the rhizospheric soil but slightly increased its measured EC and salinity. Hence, (NH₄)₂SO₄ is a more suitable candidate for the control of the nematode than NH₄Cl, which showed similar salinity level to NaCl.

In a previous report where the effect of concentration gradient of KNO₃ on horizontal migration of M. javanica J2s was studied, the juveniles moved preferentially toward the lower mineral salt concentration region (Prot, 1979). However, in our experiments, high concentrations of nitrate as KNO₃ did not considerably affect nematode infection or reproduction on the host. This is in an agreement with the results of other studies (Marks and Sayre, 1964; Ismail and Saxena, 1977).

Generally, ammonium was more effi-
Control of *Meloidogyne javanica* with N-containing salts on tomato

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**Literature Cited**


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Αποτελεσματικότητα των ανόργανων αλάτων αζώτου στην αντιμετώπιση του νηματώδη Meloidogyne javanica στην τομάτα

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Περίληψη Στην παρούσα εργασία μελετήθηκε η επίδραση διαφόρων ανόργανων αλάτων αζώτου και του χλωριούχου νατρίου σε συνδυασμό με αυξανόμενα επίπεδα ηλεκτρικής αγωγιμότητας (ECs 2, 4, 6 and 8 mhhos/cm) στον νηματώδη Meloidogyne javanica και η αλληλεπίδραση αυτών των παραγόντων σε φυτά τομάτας cv. GS12, σε συνθήκες εργαστηρίου και στο θερμοκήπιο. Το χλωριούχο αμμώνιο (NH₄Cl) και το θειικό αμμώνιο [(NH₄)₂SO₄] ήταν περισσότερο αποτελεσματικά από το νιτρικό αμμώνιο (NH₄NO₃), το οποίο ήταν πιο δραστικό από το νιτρικό κάλιο (NH₄NO₃) και το χλωριούχο νάτριο (NaCl) στην καταστολή του M. javanica προκαλώντας μείωση των όγκων στις ρίζες και στην αναπαραγωγή των νηματωδών στα φυτά της τομάτας. Σε συνθήκες θερμοκηπίου, οι ελάχιστες σημαντικές τιμές του δείκτη παρουσίας όγκων στις ρίζες ήταν 1,60, 2,04, 2,30 και 3,30, στις επεμβάσεις με NH₄Cl, (NH₄)₂SO₄, NH₄NO₃ και KNO₃ αντίστοιχα, ενώ η μέγιστη τιμή (4,01) παρατηρήθηκε στο NaCl και δεν διέφερε στατιστικά σημαντικά από το μάρτυρα (4,92). Επιπλέον, παρατηρήθηκε σημαντική αύξηση στην ανάπτυξη των φυτών τομάτας και στην περιεκτικότητα τους σε πρωτεϊνη στις επεμβάσεις με (NH₄)₂SO₄ και NH₄NO₃, αντίθετα στην επέμβαση με NaCl, το ξηρό βάρος των φυτών και η περιεκτικότητα σε πρωτεϊνη ήταν μειωμένη λόγω της αλατότητας σε σχέση με τον μάρτυρα. Οι υψηλότερες τιμές ηλεκτρικής αγωγιμότητας στα διαλύματα των αλάτων αζώτου δεν επηρέασαν το pH του εδάφους της ριζόσφαιρας αλλά αύξησαν ελαφρώς την εκτίμηση της αγωγιμότητας και την αλατότητα του. Συνεπώς, το (NH₄)₂SO₄ είναι καταλληλότερο άλας αζώτου από το NH₄Cl για την αποτελεσματική αντιμετώπιση του νηματώδη M. javanica όταν το νερό άρδευσης είναι περιοριστικός παράγοντας με επίπεδα αλατότητας ανάλογα με αυτά του NaCl. Η χρήση αμμωνιακών αλάτων, ειδικά (NH₄)₂SO₄ και NH₄Cl, από μόνη της ή σε συνδυασμό με άλλα μέτρα αντιμετώπισης μπορεί να είναι αποτελεσματική στην αντιμετώπιση του νηματώδη M. javanica.
