SALT SUPPRESSION OF *MELOIDOGYNE JAVANICA* ON TOMATO

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Summary. The influence of ammonium chloride, potassium nitrate and sodium chloride, and inoculation with the root-knot nematode *Meloidogyne javanica*, were evaluated at two levels of electrical conductivity (EC, 4 and 8) in two tomato cultivars (GS12, root-knot susceptible, and Asala, root-knot resistant). Ammonium chloride was more effective than potassium nitrate at both ECs in causing mortality of second-stage juveniles and reducing nematode reproduction (eggs/g fresh root) and root galling. Sodium chloride and potassium nitrate caused significantly greater reductions of shoot and root fresh weights of tomato than ammonium chloride. Thus, ammonium chloride could perhaps be used as an effective and environmentally acceptable control option for *M. javanica* on tomato.

Key words: Control, host resistance, root-knot nematode, *Solanum lycopersicum*.

Root-knot nematodes (*Meloidogyne* spp.) are the most economically important group of plant-parasitic nematodes worldwide, attacking nearly every crop grown (Sasser and Freckman, 1987). In Jordan, average annual losses of irrigated vegetable crops in the Jordan Valley due to root-knot nematodes are estimated at nearly 15% (Abu-Gharbieh, 1994).

Salts may function as stimulant or depressant factors on various physiological functions of the plant, especially when combined with other stressful agents such as nematodes (Edongali and Ferris, 1982). Gradients of salts of the specific ion repellents (NH_4^+, K^+, Cl^-, and NO_3^-) for *Meloidogyne incognita* (Kofoid *et* White) Chitw. have been demonstrated to shield tomato roots from infection in soil (Marks and Sayre, 1964; Ismail and Saxena, 1977; Edongali and Ferris, 1982; Castro *et al*., 1991). Some of these ions (NH_4^+, K^+, and NO_3^-) are beneficial to plant growth, and the results suggest that a new environmentally acceptable means of plant protection is possible (Castro *et al*., 1991). The objectives of this study were to investigate the effects of various levels of NH_4Cl, KNO_3 and NaCl, at two electrical conductivity (EC) levels, on the root-knot nematode, *M. javanica* (Treub) Chitw., and their interactions on the growth of two tomato cultivars, one susceptible and one resistant to *M. javanica*.

MATERIALS AND METHODS

Mortality and infectivity bioassays

Analytical reagent grade of the three salts NH_4Cl, KNO_3 and NaCl were used. The electrical conductivities of each of the salt solutions used were 4 (EC4) and 8 (EC8) mmhos/cm and were achieved by dissolving 2.138 and 4.277 g/l NH_4Cl, 4.041 and 8.082 g/l KNO_3, or 2.337 and 4.674 g/l NaCl, respectively, in distilled water.

The nematode population used in the study was derived from a single egg-mass collected from an eggplant field in Dier Alla in the Jordan Valley. It had previously been identified as *M. javanica* through morphological and host preference characteristics and confirmed by species-specific SCAR-PCR (Karajeh, 2004).

Mortality test. One hundred two-day-old 2nd stage juveniles (J_2), hatched from eggs surface-sterilized in 0.5% NaOCl, were picked and transferred to a small Petri dish containing 5 ml of each solution; distilled water served as control. Each treatment was replicated three times. The bioassay was conducted in the dark at room temperature (25 °C). The J_2s were examined after 24 hours and those that failed to react to probing with a fine bristle were considered dead. Mortality bioassay data was converted to percentage J_2 mortality and corrected utilizing Abbott's correction formula (Abbott, 1925). The mortality assay was performed three times.

Infectivity test. Two-week-old tomato seedlings (averaging 5 cm tall) of the *M. javanica* susceptible cv. GS12 were transferred into 100 ml plastic cups with the base removed and sealed with a fine tissue paper. The cups were filled with a sterilized mixture (2:1) of peat and perlite, planted with one seedling per cup and placed on Petri dishes containing 10 ml of saline solutions prepared from the three salts at EC4 and EC8. Each seedling was inoculated with 100 two-day-old J_2s, hatched from eggs surface-sterilized with 0.5% NaOCl, by picking and transferring the J_2s into the saline solution in each Petri dish. Distilled water served as a control. Each treatment was replicated three times. In order to infect the seedling root system, the J_2s would have to

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move in the saline solution or distilled water through the tissue paper to the roots. The plants were regularly irrigated with sterile distilled water and the saline solutions under test were replaced by sterile distilled water after 7 days. Thereafter, the treated and control plants were maintained in a growth chamber at 25 °C and 16/8 hour light/dark regime for 3 weeks. The plants were then up-rooted and their roots were evaluated for galling index to assess the infectivity of the J_2s. The galling index was evaluated according to the scale: 0 = no galling, and 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = over 100 galls (Taylor and Sasser, 1978). The infectivity assay was performed twice.

Pot Experiment

To determine the effect of the interaction of *M. javanica* and the variable levels (EC4 and EC8) of the three salts on the growth of two tomato cultivars, one-month-old seedlings (about 15 cm tall) of the cultivar resistant to *M. javanica*, cv. Asala, and the susceptible cv. GS12 were planted in 2 dm^3 pots filled with 1.5 kg of a mixture (1:2) of water-washed sand and a non-sodic, non-saline clay loam soil (EC 0.8 mmhos/cm, pH 7.9, 0.8% organic matter, 1.03 mg/g total nitrogen, and 38% $CaCO_3$) that had been previously pasteurized at 85 °C for 5 days. One week after transplanting, the levels of electrical conductivities (EC4 and EC8) were achieved and maintained by weekly irrigation of the pot soil with saline solutions to field capacity level for eight consecutive weeks, until one week before the end of the experiment. For the control treatments, the same procedure was followed except that tap water replaced the salt solution.

Each of the six replicated tomato seedlings (one plant per pot) was inoculated with 3000 nematode eggs. The inoculation was performed one week after salination (two weeks after transplanting) by pouring the egg suspension of the nematode into three holes made in the soil around the seedling. Non-inoculated tomato plants were used as controls. The tomato plants were transferred to a greenhouse (25 ± 5 °C air temperature and 12 h day) and kept without fertilization. All treatments were arranged in a randomized complete block design of 2×2×3×2 factorial design (2 cultivars, *M. javanica* (present or absent), 3 treatments (2 ECs level and a control) with six replicates. Sixty days after inoculation, plants were removed from pots and the roots were carefully washed to remove soil particles. Shoot and root fresh weights were recorded. The galling index was evaluated as previously described. Each plant root system was finely chopped and blended in a 0.5% NaOCl solution to extract eggs (Hussey and Barker, 1973). The resulting suspension was poured through a 75 µm sieve nested onto a 26 µm sieve. The eggs, collected on the 26 µm sieve, were counted and their numbers expressed per g of fresh roots.

Statistical analysis

Data from the two bioassays and pot experiment were statistically analyzed using the analysis of variance (ANOVA) procedure of SAS (SAS Institute Inc., Cary, NC). Significance of interactions was tested at $P = 0.05$. Fisher's protected least significance difference test (FLSD) was used for mean separation at the 0.05 probability level (Steel *et al.*, 1997).

RESULTS

Mortality and Infectivity Bioassays. Corrected percentage mortality of J_2s was greater ($P \leq 0.05$) for NH_4Cl at EC8 (95.9%) followed by EC4 (86.6%) than that of the other treatments at both ECs. Mortality was 46.4 and 54.6% for KNO_3 at EC4 and EC8, respectively, and was not different from the control for NaCl at EC4 (Table I). Corrected mortality percentages of J_2s were significantly higher at EC8 than at EC4 regardless of the salt type. There was no obvious effect for NaCl at EC4 on the activity of J_2s (Table I), reflected in the high galling index values (4.0 and 5.0, respectively) recorded for NaCl at EC4 and the control. The least galling occurred with NH_4Cl at EC8 (0.3) and NH_4Cl at EC4 (0.7). The galling indices in the KNO_3 and NaCl treatments, regardless of EC value, were greater than those of NH_4Cl treatments. The infectivity of J_2s did not differ significantly at EC8 from that at EC4, regardless of the salt type, as indirectly indicated by the galling index (Table I).

Pot Experiment. *Meloidogyne javanica* reproduction and root galling were affected by salt and EC (Table II). The effects of the nematode also interacted with those of salt and EC on the fresh weights of plant shoots and roots. The presence or absence of salt affected the fresh weights, root galling and nematode reproduction, while EC levels affected shoot fresh weight and nematode reproduction. The cultivar × salt interaction affected nematode reproduction while the cultivar × EC interaction affected all of the variables measured. There were no effects on the fresh weights of the interactions for cultivar × nematode × salt or cultivar × nematode × salt × EC (Table II).

Shoot and root fresh weights were generally greater (≤ 20% increases) in the controls than in any of the salt treatments except for NH_4Cl at EC4 (Fig. 1A and 1B). The weight reduction was considerable for NH_4Cl at EC8, NaCl at EC8 and KNO_3 at both ECs compared with the control, especially in the susceptible cv. GS12 (Fig. 1A).

Root galling was significantly less ($P \leq 0.05$) in the salt treatments than in the inoculated control except in the NaCl treatment at EC4 (Fig. 2A). Obvious differences were found in root galling between cvs. Asala and GS12 at both EC4 and EC8 for the NH_4Cl treatment (Fig. 2A).

There was a significant reduction in nematode reproduction (expressed as number of eggs/g fresh root) in NH_4Cl and KNO_3 salt treatments compared with the control, with the greatest reduction observed in the NH_4Cl treatment (Fig. 2B).

Table I. Mortality and infectivity (measured by galling index) of second-stage juveniles of *Meloidogyne javanica* after exposure to salts at two electrical conductivity (EC) levels.

Salt	EC[w] (mmhos/cm)	Corrected Mortality %[x]	Galling index (0-5)[y]
Untreated (control)	0	0[z] f	5.0 a
NH_4Cl	4	86.6 b	0.7 d
	8	95.9 a	0.3 d
NaCl	4	3.1 f	4.0 ab
	8	21.6 e	3.3 bc
KNO_3	4	46.4 d	3.3 bc
	8	54.6 c	2.3 c
FLSD ($P \leq 0.05$)		7.3	1.2

Data are means of three replicates in the mortality bioassay repeated three times and the infectivity bioassay repeated twice. Means compared using Fisher's protected least significance difference test, FLSD ($P \leq 0.05$).

[w] EC: electrical conductivity.

[x] Corrected mortality % = (mortality in treatment – mortality in control) × 100% / (100 - mortality in control) (Abbott, 1925).

[y] Galling index: 0 = no galling; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; and 5 = over 100 galls (Taylor and Sasser, 1978).

[z] Means with the same lower case letter within columns are not significantly different according to Fisher's protected least significance difference test, FLSD ($P \leq 0.05$).

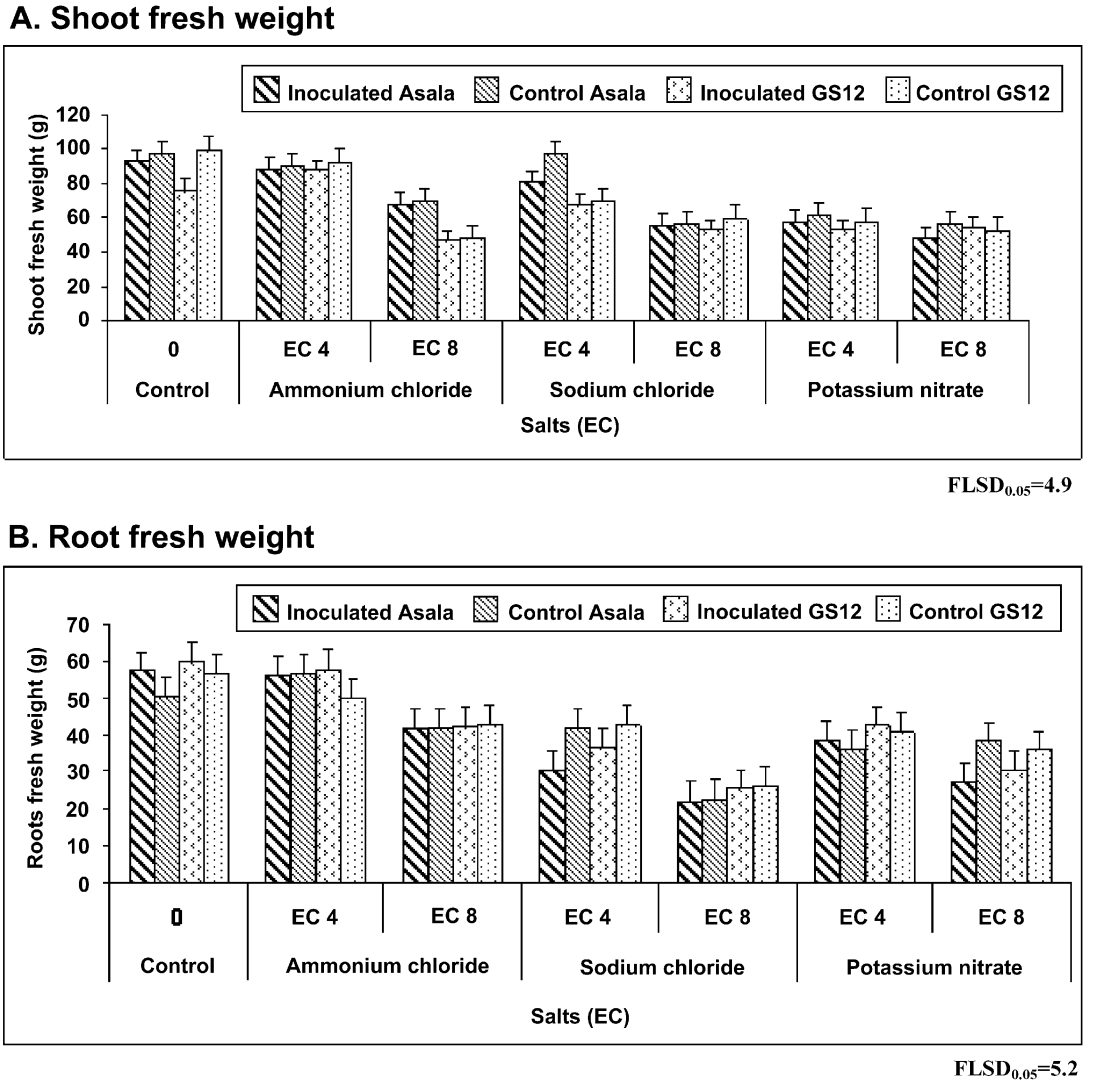

Fig. 1. Effect of salts at different electrical conductivity (EC) levels on shoot (A) and root fresh weights (B) of the two tomato cultivars (Asala resistant and GS21 susceptible) inoculated with *Meloidogyne javanica*.

Table II. Main and interaction effects (*P* values) on plant fresh weights, root galling and nematode reproduction for two tomato cultivars grown in pots treated with different salts at two levels of electrical conductivity (EC) with or without the root-knot nematode *Meloidogyne javanica*.

Source	Shoot fresh weight	Root fresh weight	Root galling	Nematode reproduction
Cultivar	0.0636	0.1256	0.0582	0.05335
Nematode	0.0758	0.2562	-	-
Salt	0.0242	0.0438	0.0435	0.0102
EC	0.0431	0.0863	0.1200	0.0253
Cultivar × Nematode	0.1324	0.1020	-	-
Cultivar × Salt	0.0547	0.0735	0.2305	0.0403
Cultivar × EC	0.0358	0.0122	0.0497	0.0085
Nematode × Salt	0.0125	0.0456	-	-
Nematode × EC	0.0037	0.0197	-	-
Cultivar × Nematode × Salt	0.3580	0.2258	-	-
Cultivar × Nematode × Salt × EC	0.7058	0.5201	-	-

Experiment analysis based on six replicates for NH_4Cl, NaCl and KNO_3 salts at two EC levels. *P* values ≤ 0.05 are significant.

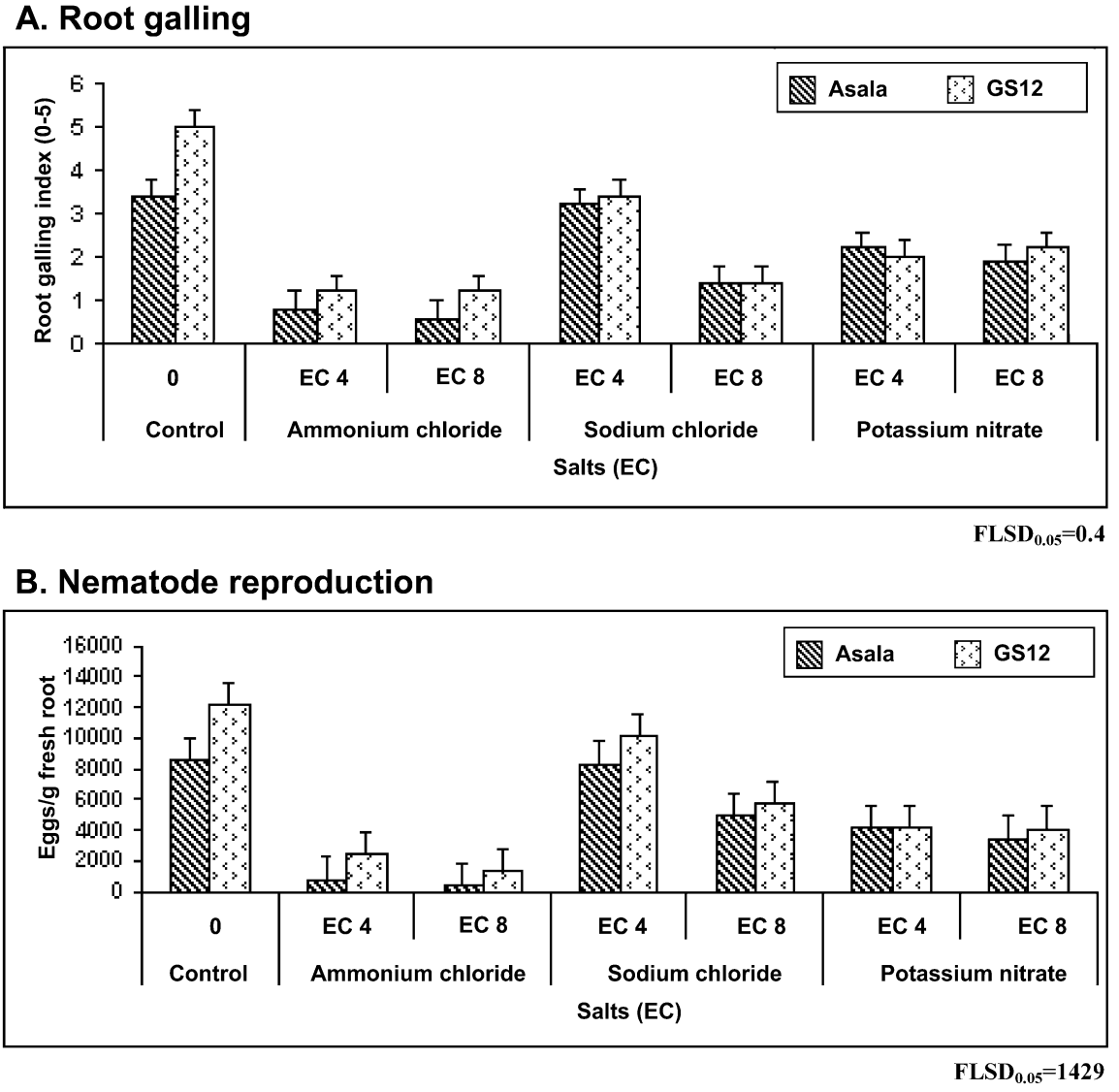

Fig. 2. Effect of different salts at two levels of electrical conductivity (EC) on root galling (A) and reproduction (B) of *M. javanica* on two tomato cultivars (Asala resistant and GS12 susceptible).

DISCUSSION

The effects of root-knot nematodes on plants grown under saline conditions are complex and horticultural measurements alone provide only a partial picture (Maggenti and Hardin, 1973). When the nematode-inoculated plants were considered, a decline in shoot and root fresh weights was observed when salt concentrations were increased from EC4 to EC8, except for potassium nitrate on shoot fresh weight. However, when inoculated and control plants were compared, the observed differences were generally not significant. Nematode damage was generally more evident at higher salinity levels, when conditions were unfavourable for plant growth (Van Gundy and Martin, 1961; Maggenti and Hardin, 1973; Edongali and Ferris, 1982). However, such conditions were also unfavorable for the nematode, resulting in lower nematode reproduction as salinity increased from EC4 to EC8. In the pot experiment, the plants were not fertilized, therefore the observed differences in growth variables between non-saline and saline conditions may have been underestimated. The effects of salts on nematodes may vary. For example, plants of cotton exposed to increasing salinity had decreased population densities of *Rotylenchulus reniformis* Linford *et* Oliveira (Heald and Heilman, 1971). Similar results were also obtained by Mashela *et al.* (1992) with citrus inoculated with *Tylenchulus semipenetrans* Cobb.

In our study, NH_4Cl at EC4 and EC8 reduced nematode reproduction by increasing J_2 mortality, apparently reducing the infectivity of J_2s and consequently reducing root galling. Reproduction of *M. incognita* on susceptible varieties also decreased with increasing salinity (Edongali and Ferris, 1982). Earlier work, conducted in sterile and non-sterile soil, suggested that application of ammonium salts had a nematicidal effect against *Pratylenchus penetrans* (Cobb) Filipjev *et* Schuurmans Stekhoven (Walker, 1971). A higher ammonium concentration in nutrient medium, in the presence and absence of excised roots, decreased the rate and total number of J_2s that hatched from dispersed eggs and egg masses (Sudirman and Webster, 1995). The higher concentrations of ammonium may have been sufficient to modify malate dehydrogenase activity (Viglierchio, 1979), and thus decrease the energy available for hatching and plant invasion processes. Dropkin *et al.* (1958) found that high salt concentrations also modify osmotic pressure sufficiently to inhibit hatching and movement of *Meloidogyne* spp. Host plant nutrition alters the degree of nematode attraction and penetration through plant roots (McClure and Viglierchio, 1966). Scott and Martin (1962) showed that treatment with different ions significantly affected the electrical potential around the root tip area where J_2 penetrated. Thus, it is possible that the relative concentration of ammonium ions may influence nematode penetration of the root through diminished attractiveness of the root tips. When a salt gradient barrier was inserted in the soil between plant roots and *M. incognita,* the juveniles were repelled by salts containing K^+ or NH_4^+ (Castro *et al.*, 1991). However, in our experiments, high concentrations of nitrate as KNO_3 did not greatly affect J_2 mortality, infectivity or nematode reproduction on the host. This is in accordance with the results of other studies (Marks and Sayre, 1964; Ismail and Saxena, 1977).

It has been hypothesized (Rodriguez-Kabana, 1986) that rates of ammonia-based fertilizer high enough to also have a nematicidal effect would probably result in phytotoxicity. However, in our experiments, plant growth was not greatly affected by the NH_4Cl treatments, with no noticeable phytotoxicity at EC4 while, at EC8, NH_4Cl caused less reduction in root growth than NaCl and KNO_3 in the resistant cultivar. Ammonium sulphate, $(NH_4)_2SO_4$, suppressed the *M. incognita* population although phytotoxicity occurred at the highest dosage, 2 g N per kg soil (D'Addabbo *et al.*, 1996). In another study, nitrogen fertilization with $(NH_4)_2SO_4$ or NH_4Cl, at similar application rates (2 g N per kg soil) to EC8, resulted in greater reduction in galling and nematode reproduction and greater increase in plant growth without any report of phytotoxicity (Akhtar *et al.*, 1998). Concerning phytotoxicity, NH_4Cl may be more preferable to use than ammonium sulphate.

Reproduction of *M. javanica* and root galling were significantly reduced by NH_4Cl compared with KNO_3 and NaCl. In a previous study, high concentrations of ammonium nitrate (NH_4NO_3) incorporated into the media of monoxenic cultures of *M. incognita* on excised tomato roots significantly inhibited giant cell formation and nematode development without affecting root growth (Orion *et al.*, 1980). Observations with scanning and transmission electron microscopy indicated that an elevated concentration of NH_4NO_3, above 400 µg/ml, inhibited giant cell formation and suppressed nematode development in the infected tomato roots (Orion *et al.*, 1995). These results support our findings. Moreover, our results indicate that ammonium rather then nitrate was mainly responsible for the suppression of nematode infection and development.

The root-knot nematode (*M. javanica*) reproduced on the two cultivars (Asala and GS12) regardless of the presence or absence of salts. Both Asala and GS12 supported nematode reproduction but root galling was greatly reduced in cv. Asala.

Preliminary checks to ascertain the effect of treatments on total phenol content of tomato roots (data not shown) revealed an increase in total phenol content of tomato roots in salted soil compared with non-salted soil, indicating that salt-stressed plants could accumulate phenols in their roots and that this may have contributed to plant resistance to nematodes. This was in agreement with Hung and Rohde (1973), who reported that *M. incognita*-resistant tomato cv. Nemared contained the highest concentration of chlorogenic acid (identified as the major phenolic compound in healthy tomato roots, concentrated in root endodermis) while

the susceptible cv. B-5 contained the lowest concentration, and Amalraj (1995), who found a proportionally higher polyphenol oxidase content in resistant potato plants after inoculation with *Globodera pallida*.

In conclusion, the laboratory and greenhouse experiments suggest that NH_4Cl could potentially be used in controlling the root-knot nematodes *M. javanica* on tomato. However, salt levels must be considered very carefully and further studies should be conducted under field conditions.

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LITERATURE CITED

Abbott W.S., 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, *18*: 265-266.

Abu-Gharbieh W.I., 1994. *Root-Knot Nematodes*, Meloidogyne *spp. in Jordan: Biology and Control*. 2nd edition, University of Jordan, Amman, Jordan, 97 pp.

Akhtar M., Siddiqui Z.A. and Mahmood I., 1998. Management of *Meloidogyne incognita* in tomato by some inorganic fertilizers. *Nematologia Mediterranea, 26*: 23-25.

Amalraj S.F., 1995. Enzyme activity associated with resistance in potato to early stages of *Globodera pallida* infection. *Nematologia Mediterranea, 23*: 199-202.

Castro C.E., McKinney H.E. and Lux S., 1991. Plant protection with inorganic ions. *Journal of Nematology, 23*: 409-413.

D'Addabbo T., Filotico A. and Sasanelli N., 1996. Effect of calcium cyanamide and other ammonia fertilizers on *Meloidogyne incognita*. *Nematologia Mediterranea, 24*: 209-214.

Dropkin V.H., Martin G.C. and Johnson R.W., 1958. Effect of osmotic concentration on hatching of some plant parasitic nematodes. *Nematologica, 3*: 115-126.

Edongali E.A. and Ferris H., 1982. Varietal response of tomato to the interaction of salinity and *Meloidogyne incognita* infection. *Journal of Nematology, 14*: 57-62.

Heald C.M. and Heilman M.D., 1971. Interaction of *Rotylenchulus reniformis*, soil salinity, and cotton. *Journal of Nematology, 71*: 149-152.

Hung C.L. and Rohde R.A., 1973. Phenol accumulation related to resistance in tomato to infection by root-knot and lesion nematodes. *Journal of Nematology, 5*: 253-258.

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

Ismail W. and Saxena K., 1977. Effect of different levels of potassium on the growth of root-knot nematode, *Meloidogyne incognita*, on tomato. *Nematologica, 23*: 263-264.

Karajeh M.R., 2004. Identification, distribution, and genetic variability of the root-knot nematodes (*Meloidogyne* spp.) in Jordan. Ph.D. Thesis, University of Jordan, Amman, Jordan, 152 pp.

Orion D., Wergin W.P. and Endo B.Y., 1980. Inhibition of syncytia formation and root-knot nematode development on cultures of excised tomato roots. *Journal of Nematology, 12*: 196-203.

Orion D., Wergin W.P. and Chitwood D.J., 1995. Root cortical cell spherical bodies associated with an induced resistance reaction in monoxenic cultures of *Meloidogyne incognita. Journal of Nematology, 27*: 320-327.

Maggenti A.R. and Hardin A., 1973. The effect of soil salinity and *Meloidogyne javanica* on tomato. *Journal of Nematology, 5*: 231-234.

Marks C.F. and Sayre R.M., 1964. The effect of potassium on the rate of development of the root-knot nematodes *Meloidogyne incognita, M. javanica* and *M. hapla. Nematologica, 10*: 323-327.

Mashela P., Duncan L.W., Graham J.H. and McSorley R., 1992. Leaching soluble salts increases population densities of *Tylenchulus semipenetrans*. *Journal of Nematology, 24*: 103-108.

McClure M.A. and Viglierchio D.R., 1966. Penetration of *Meloidogyne incognita* in relation to growth and nutrition of sterile, excised cucumber roots. *Nematologica, 12*: 237-247.

Rodriguez-Kabana R., 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. *Journal of Nematology, 18*: 129-135.

Sasser J.N. and Freckman D., 1987. A world perspective on nematology: the role of the society. Pp. 7-14. *In*: Vistas on Nematology (Veech J. and Dickson D., eds). Society of Nematologists, Hyattsville, Maryland, USA.

Scott B.I. and Martin D.W., 1962. Bioelectric fields of bean roots and their relation to salt accumulation. *Australian Journal of Biological Sciences, 15*: 83-100.

Steel R.D., Torrie J.H. and Dickey D.A., 1997. *Principles and Procedures of Statistics: A Biometrical Approach*. 3rd edition, McGraw-Hill Inc., New York, USA. 612 pp.

Sudirman I. and Webster J.M., 1995. Effect of ammonium ions on egg hatching and second-stage juveniles of *Meloidogyne incognita* in axenic tomato root culture. *Journal of Nematology, 27*: 346-352.

Taylor A.L. and Sasser J.N., 1978. *Biology, Identification and Control of Root-Knot Nematodes (*Meloidogyne *spp.)*. North Carolina State University Graphic, Raleigh, NC, USA, 111 pp.

Van Gundy S.D. and Martin J.P., 1961. Influence of *Tylenchulus semipenetrans* on growth and chemical composition of sweet orange seedlings in soils of various exchangeable cation ratios. *Phytopathology, 51*: 146-151.

Viglierchio D.R., 1979. Selected aspects of root-knot nematode physiology. Pp. 114-154. *In*: Root-Knot Nematodes (*Meloidogyne* species) (Lamberti L. and Taylor C.E., eds). Academic Press, New York, USA.

Walker J.T., 1971. Populations of *Pratylenchus penetrans* relative to decomposing nitrogenous soil amendments. *Journal of Nematology, 3*: 43-49.

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