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Field Utilization of Nitrogen Fertilizers for Controlling Root-knot Nematode and Improving Growth and Yield of Cucumber

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Abstract Efficacy of nitrogen salts at 2, 4, and 8 mmhos/cm electrical conductivity (EC) on the host-pathogen relationship of root-knot nematode (*Meloidogyne javanica*) and cucumber (*Cucumis sativus*) was investigated and compared with the effects of nematicides at 2 locations. Root galling of cucumber roots caused by *M. javanica* was more effectively suppressed using ammonium-containing salts: NH_4Cl, $(NH_4)_2HPO_4$ and $(NH_4)_2SO_4$ at EC4 and EC8 than at EC2 and in a similar effect level of the nematicide, oxamyl or ethoprophos, and the reduction was accompanied by a reduction in the nematode final population number. At higher EC levels, NH_4Cl followed by $(NH_4)_2SO_4$ led to an increase of salinity up to about 1750 and 1245 mg/kg, respectively, without affecting cucumber growth and yield. Diammonium phosphate was superior over the ammonium salts in increasing cucumber growth and yield despite root-knot infection. Use of nitrogen salts could control *M. javanica* and improve growth and yield of cucumber under field conditions.

Keywords Ammonium salts, *Cucumis sativus*, *Meloidogyne javanica*, Plant-nematode interaction, Management

1. Introduction

Cucumber (*Cucumis sativus* L.) grows on most continents worldwide and is a common crop cultivated in irrigated areas of Jordan. Root-knot nematodes (RKN; *Meloidogyne* Goldi 1892 - RKNs) are a group of important plant-parasitic nematodes attacking higher plants[30; 33; 34], causing great crop losses[35] and reducing quality of produce[4].

Three major *Meloidogyne* species (*M. javanica* [Treub] Chitw., *M. incognita* [Kofoid et White] Chitw., race 1 and 2 and *M. arenaria* [Neal] Chitw., race 2) were reported in Jordan, with *M. javanica* being the most widely distributed in Jordan[2; 16] causing considerable losses in yield of irrigated vegetable crops[1].

Many physiological functions of the plant could be influenced by inorganic salts, especially when in an interaction with stress such as infection by nematodes[9; 32]. Some ions (NH_4^+, K^+, NO_3^- and Cl^-) were effective in reducing *M. incognita* infection of cucumber roots[6; 12; 24]. Among these NH_4^+, K^+, and NO_3^- increased plant growth[6; 37]. Application of fertilizers negatively influences nematode populations indirectly by decreasing nematode feeding or by providing adequate nutrition for plants to compensate loss due to nematode feeding[26]. Nutrient deficiency could increase susceptibility of plants to nematodes[28]. Direct effects of fertilizers on nematodes may alter their behavior and reproduction resulting in changes in population[8; 17; 19]. Karajeh & Al-Nasir[18] reported that under *in vitro* growth chamber and greenhouse conditions, egg-hatch and second-stage juvenile viability of *M. javanica* were reduced when ammonium chloride (NH_4Cl), ammonium sulfate $(NH_4)_2SO_4$) and ammonium nitrate (NH_4NO_3) were used at electrical conductivity (EC) levels of 6 and 8 mmhos·cm^{-1} accompanied with reduction in cucumber root galling. The diammonium phosphate $(NH_4)_2HPO_4$ caused the greatest reduction in root galling to <2.5 and was superior in increasing plant growth. As a salt, NaCl caused a reduction in both nematode infection and root growth especially at higher EC levels due to its salinity effect.

This study investigates the effects of the nitrogen containing fertilizers; NH_4Cl, $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ on growth and yield of cucumber and its interaction with the RKN, *M. javanica* under two field conditions of Jordan.

2. Materials and Methods

Two *M. javanica*-infested fields were selected; one was located in the Ghor Safi region of southern Ghors, with semi-arid subtropical climate conditions (about 80 mm of annual rainfall and silty loam soil, at 418 m below sea level).

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The other was located in the Karak Valley region with semi-arid conditions (about 200 mm of annual rainfall and clay loam soil, at about 200 m above sea level) of Jordan. Drip irrigation was used on both sites. The field experiments were conducted during mid-September 2012 to mid-March 2013 and mid-February to mid-July 2013 cucumber growing seasons in Ghor Safi and Karak Valley, respectively. Field populations of RKNs of the sites were identified as *M. javanica* morphologically by observing the perineal patterns of females and morphometrically by measuring lengths of second-stage juveniles[5] and confirmed by *Meloidogyne* species-specific SCAR-PCR test[15].

Seeds of cucumber cv. Hayel were sown into holes (2 seeds per a hole) made into black plastic mulch-covered rows with 80 cm width and 150 cm spacing between rows and then irrigated. The seedling emerged after 4 days. The experiment was arranged in a randomized complete block design and each treatment (5 plants per plot) was replicated times. When plants were 2 weeks old the treatments applied using analytical reagent grade nitrogen salts were: NH_4Cl, $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$. Electrical conductivity for each salt solution was 2 (EC2), 4 (EC4), and 8 (EC8) mmhos·cm^{-1} achieved by dissolving 1.067, 2.138, and 4.277 g·L^{-1} NH_4Cl; 2.633, 5.276 and 10.553 g·L^{-1} $(NH_4)_2SO_4$; and 2.51, 5.14, and 9.85 g·L^{-1} $(NH_4)_2HPO_4$, respectively, in water. Nematicides Ethoprophos, 20% (Nemacap® 200EC, Chemvet, Jordan) or Vydate® 24SL (Dupont, Delaware, USA) was added to the rhizospheric soil at manufacture's recommended high application rate in Ghor Safi and Karak Valley experiments, respectively. Untreated check (control) plants were irrigated with water only.

Treatments were repeated twice at 2 week-intervals from the first application for one month later. Drip irrigation, weed control, air-borne pest control and fruit harvest were according to Werner[40]. Accumulated amount of fruit yield per a treatment was recorded from 4 harvests per plots. Random samples of the fifth leaf from plant top (four leaves/ a treatment) and moderate-in-size cucumber fruits were taken at the middle of the growing season for further analyses in the laboratory. Representative random samples of oven dried leaves and fruits were finely grounded and analyzed for nitrogen, potassium and phosphorus content[29]. Chlorophyll content was determined by taking according to Hamid & Jawaid[14] with some modification (100% rather than 80% acetone was used).

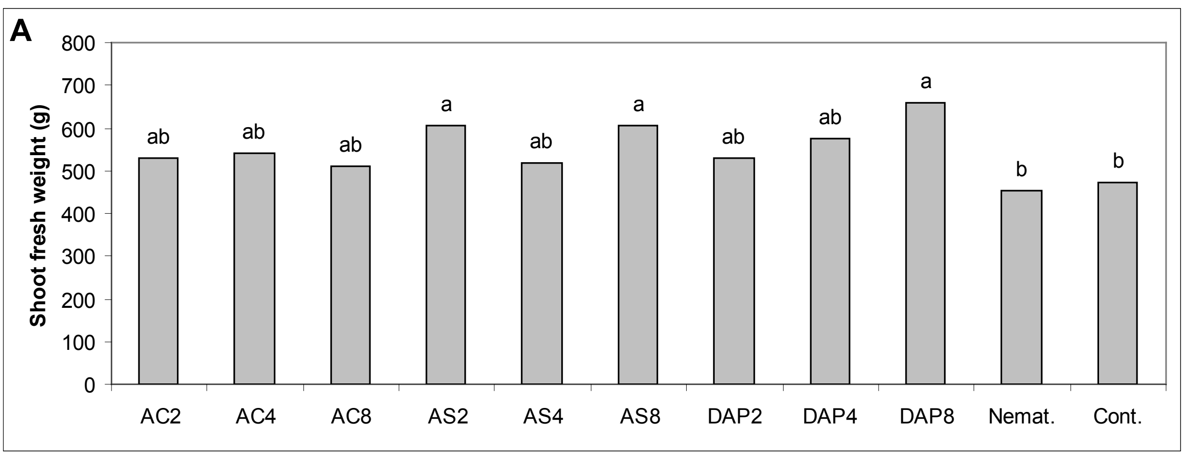
At the end of the experiment, 10 weeks from the date of seed germination, cucumber plants were up-rooted. Shoot and root fresh and dry weights were taken. Root galling index was evaluated according to a scale where: 0: no galling; 1: 1–2; 2: 3–10; 3: 11–30; 4: 31–100 and 5 over 100 galls[38]. The amount of total phenolics in roots was determined with the Folin–Ciocalteu reagent procedure[23; 25]. Representative rhizospheric soils were collected from treatments for nematode extraction using Baermann tray method[41] to estimate final nematode population. After sieving and grinding 10 g of oven dried rhizospheric soil was dissolved in distilled water, and soil pH, EC and salinity measured using PCSTestr35 (Eutech Instruments, Illinois, USA).

Statistical analysis

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

3. Results

The application of $(NH_4)_2SO_4$ at EC2 and 8 and $(NH_4)_2HPO_4$) at EC8 produced an increase in cucumber shoot fresh weight over the untreated control (Fig. 1A). Both $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ at the higher EC produced a higher root dry weight than the untreated control (Fig. 1B). The NH_4Cl; $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ at EC8 and $(NH_4)_2HPO_4$ at EC4 produced the highest fruit yield compared to the nematicide and the control (Fig. 1C).



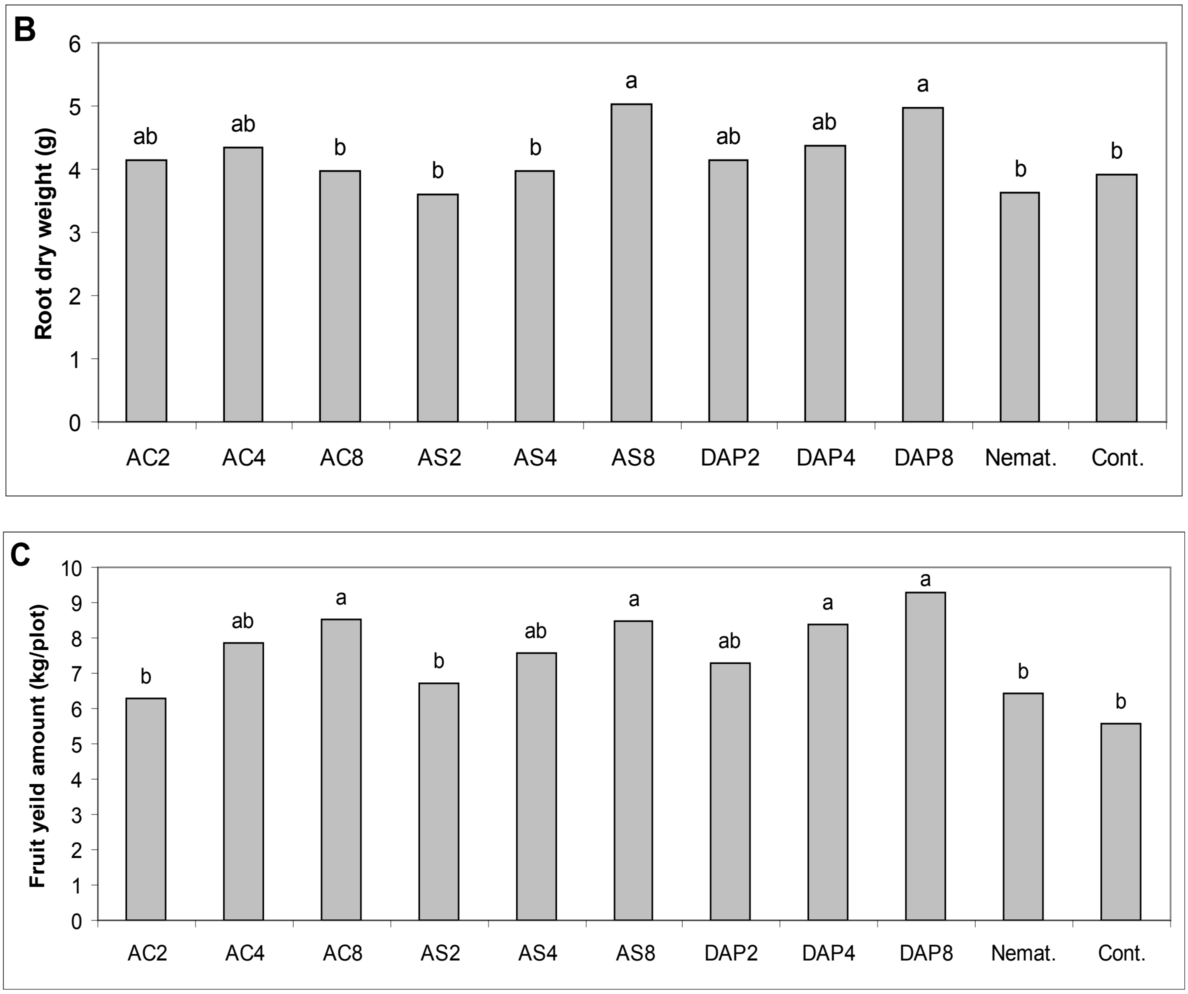
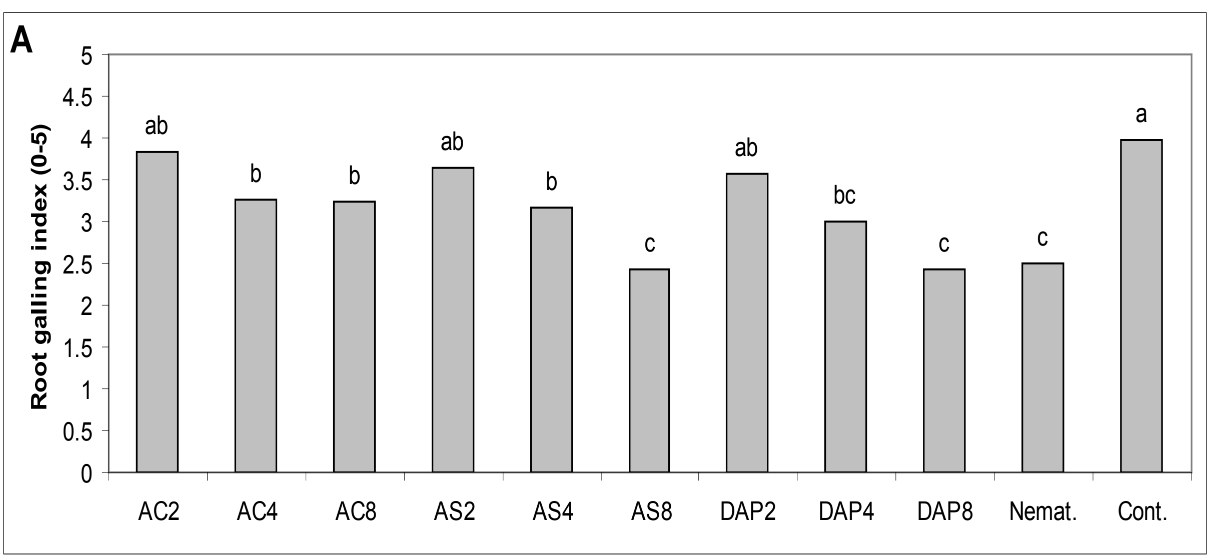


Figure 1. Effects of nitrogen salts; AC: ammonium chloride, AS: ammonium sulfate, and DAP: Diammonium phosphate at three levels of electrical conductivity (EC); 2, 4 and 8 mmhos/cm, Nemat.: Nemacap or Vydate, and cont.: untreated control on shoot fresh (in A) and root (in B) dry weights and fruit yield (in C) of cucumber infected with the root-knot nematode (*Meloidogyne javanica*) under field conditions

Root galling was reduced due to treatment with NH_4Cl, $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ at the 4 and 8 EC levels and were similar to the nematicide (Fig. 2A); there was also a reduction in nematode final population (Fig. 2B).



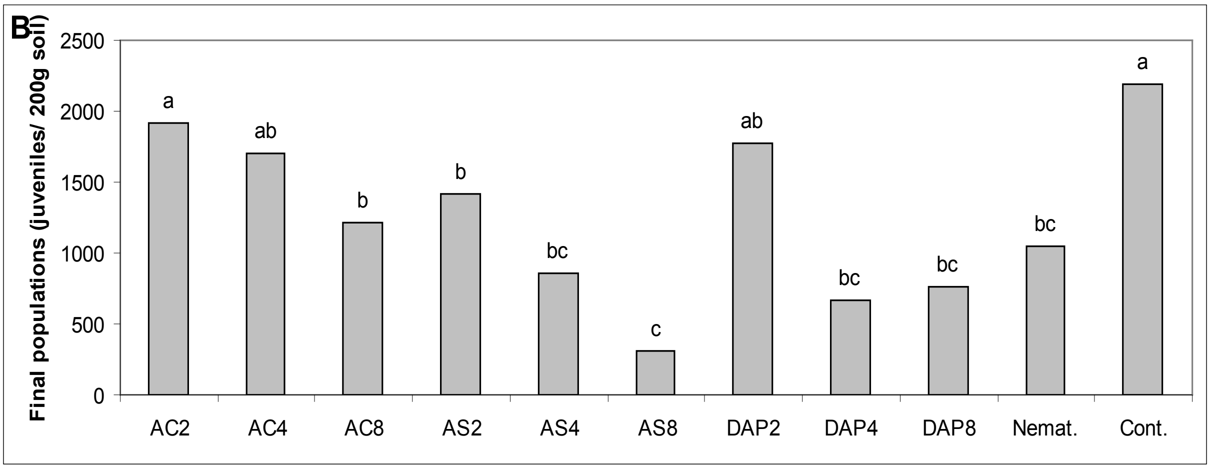
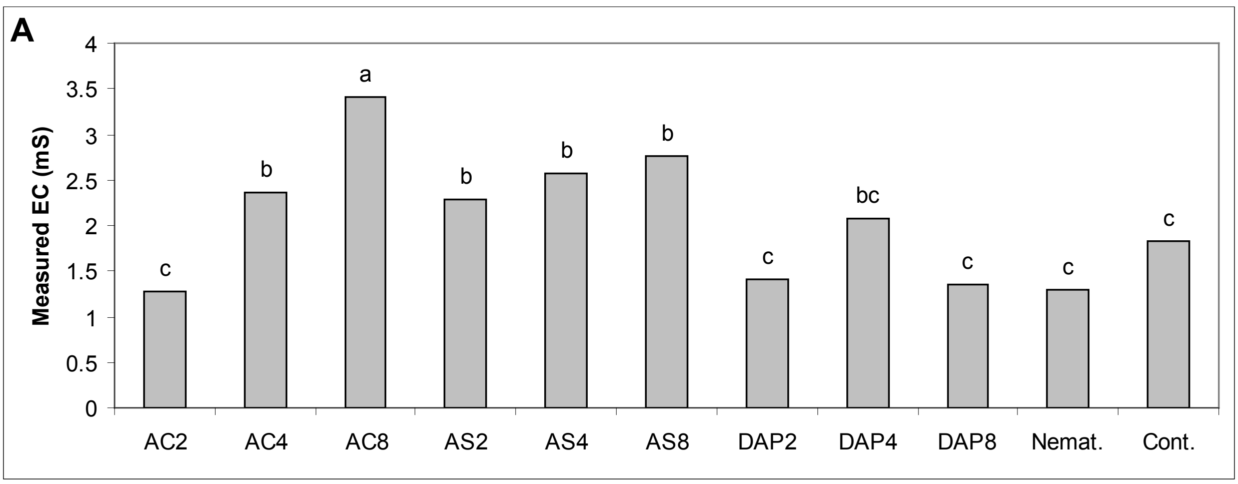


Figure 2. Effects of nitrogen salts; AC: ammonium chloride, AS: ammonium sulfate, and DAP: Diammonium phosphate at three levels of electrical conductivity (EC); 2, 4 and 8 mmhos/cm, Nemat.: Nemacap or Vydate, and cont.: untreated control) on root-galling index (in A) of cucumber infected with the root-knot nematode (*Meloidogyne javanica*) and the nematode final population number (in B) under field conditions

Application of NH_4Cl at EC8 increased the soil EC and followed by the same salt at EC4 and $(NH_4)_2SO_4$ at EC4 and EC8 which were different in their values from the untreated control (Fig. 3A, 3B).



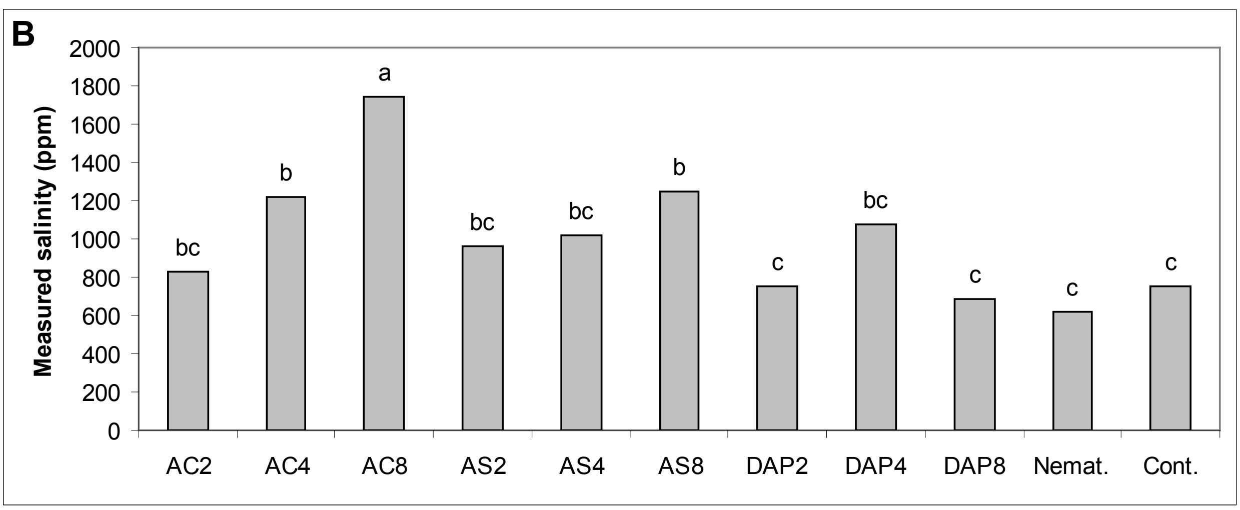


Figure 3. Effects of nitrogen salts; AC: ammonium chloride, AS: ammonium sulfate, and DAP: Diammonium phosphate at three levels of electrical conductivity (EC); 2, 4 and 8 mmhos/cm, Nemat.: Nemacap or Vydate, and cont.: untreated control) on measured EC (in A), and salinity (in B) of the rhizospheric soil of cucumber infected with the root-knot nematode (*Meloidogyne javanica*) under field conditions

Table 1. Main and interaction effects of *Meloidogyne javanica*-infested field, salt and electrical conductivity (EC) on cucumber growth, root galling, final nematode population number, chlorophyll content, root total phenol content, soil pH, EC and salinity, and nitrogen, phosphorus and potassium contents of leaves and fruit

Source	SFW[a]	RFW	RDW	Yld	GI	Pf	Chl	Phen	pH	EC	Sal	N frt	P frt	K frt
Salt (S)	0.550[b]	0.860	0.693	0.764	0.774	0.417	0.024	0.789	0.098	0.028	0.288	0.019	0.001	0.045
EC (E)	0.712	0.312	0.321	0.326	0.526	0.154	0.639	0.908	0.675	0.976	0.043	0.661	0.031	0.008
Field (F)	0.000	0.045	0.033	0.000	0.024	0.111	0.001	0.067	0.124	0.120	0.326	0.226	0.000	0.450
S × E	0.715	0.781	0.264	0.996	0.988	0.721	0.011	0.983	0.614	0.434	0.251	0.138	0.163	0.002
S × F	0.600	0.961	0.462	0.884	0.544	0.750	0.038	0.932	0.465	0.845	0.945	0.005	0.005	0.101
F × E	0.730	0.699	0.822	0.481	0.585	0.490	0.249	0.924	0.235	0.145	0.558	0.033	0.007	0.119
S × E × F	0.910	0.197	0.214	0.846	0.897	0.896	0.010	0.991	0.877	0.234	0.500	0.175	0.044	0.235

[a] PFW: plant fresh weight; SFW: shoot fresh weight; RFW: root fresh weight; RDW: root dry weight; Yld: fruit yield, GI: root galling index, Pf: final nematode population; Chl: chlorophyll content; Phen: root total phenol content; Sal: salinity; N lf: leaf nitrogen; N frt: fruit nitrogen; P lf: leaf phosphorus; P frt: fruit phosphorus; K lf: leaf potassium, and K frt: fruit potassium contents.
[b] Probability values ≤0.050 are significant.

Table 2. Simple correlations among cucumber growth parameters, root galling, final nematode population, chlorophyll content, root total phenol content, measured soil pH, EC and salinity, and on nitrogen, phosphorus and potassium contents of leaves and fruit

	SFW[a]	RFW	RDW	Yld	GI	Pf	Chl	Phen	pH	EC	Sal	N frt	P frt
RFW	-0.29												
RDW	-0.29	0.66**											
Yld	0.93**	0.36	0.22										
GI	-0.55**	-0.35	-0.16	-0.58**									
Pf	-0.48*	0.06	-0.01	-0.51**	-0.01								
Chl	-0.39*	0.53**	0.33	0.47*	-0.25	0.21							
Phen	0.64**	-0.11	0.03	0.66**	0.36	-0.15	-0.41*						
pH	-0.19	-0.16	-0.18	-0.16	0.24	0.33	-0.22	-0.45*					
EC	0.13	0.06	-0.04	0.05	-0.06	-0.18	0.01	0.39*	-0.75**				
Sal	-0.01	0.11	0.18	0.12	0.06	-0.24	-0.33	0.22	-0.12	0.21			
N frt	-0.06	-0.11	-0.15	-0.10	-0.16	0.00	0.10	-0.29	0.10	-0.23	-0.13		
P frt	0.75**	-0.60**	-0.53**	0.79**	0.55**	-0.21	-0.47*	0.39*	0.13	-0.18	-0.14	0.30	
K frt	-0.82**	0.24	0.17	0.82**	-0.58**	0.35	0.38	-0.64**	0.12	-0.07	0.02	0.50**	-0.52**

*significant at 0.05 or ** significant at 0.01 probability level.
[a] SFW: shoot fresh weight; RFW: root fresh weight; RDW: root dry weight; Yld: fruit yield; GI: root galling index; Pf: final nematode population; Chl: chlorophyll content; Phen: root total phenol content; Sal: salinity; N frt: fruit nitrogen, P frt: fruit phosphorus, and K frt: fruit potassium content.

Field, either in Ghor Safi or Karak Valley, as a main factor had significantly affected shoot and root fresh and dry weights, yield, root galling index, chlorophyll content, and potassium content of cucumber fruits. Nitrogen salt as a main factor had significantly ($P \leq 0.05$) affected chlorophyll content, measured soil EC, and nitrogen, phosphorus and potassium contents of cucumber fruits. Electrical conductivity level as a main factor had significantly affected soil salinity, and fruit content of phosphorus and potassium (Table 1). Chlorophyll content was greatly affected by Salt X EC, Salt X Field and Salt X EC X Field interactions. Fruit nitrogen content was greatly affected by Salt X Field and Field X EC interactions and potassium content was affected by Salt X EC interaction (Table 1).

Significantly, cucumber fruit yield was positively correlated with shoot fresh weight, chlorophyll content, root total phenol content, and fruit phosphorus and potassium contents but was negatively correlated with root galling index and the final population of *M. javanica* (Table 2).

4. Discussion

Use of chemicals to control phytoparasitic nematodes is expensive, can damage the environment and suppress populations of beneficial antagonistic micro-organisms in soils. Under these field conditions, root galling of cucumber roots caused by *M. javanica* was better suppressed with NH_4Cl, $(NH_4)_2HPO_4$ and $(NH_4)_2SO_4$ at in a similar manner to nematicide oxamyl or ethoprophos and the reduction was accompanied with a reduction in nematode final population. Under growth room conditions, egg-hatching and J2 viability of *M. javanica* were greatly reduced when NH_4Cl, $(NH_4)_2SO_4$ and NH_4NO_3 used at elevated levels of EC, and there was a reduction in rate of nematode infection after use of NH_4Cl, $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$ but KNO_3 and NH_4NO_3 resulted in moderate nematode infection on cucumber under greenhouse conditions[18]. Numbers of hatched eggs of *M. incognita* decreased with increasing ammonium concentrations by 15 days of incubation[36]. Dropkin *et al.* (8) found that high salt concentration can modify osmotic pressure sufficiently to inhibit egg-hatching and J2 movement of *Meloidogyne* spp. The NaCl reduced in nematode infection and root growth, especially at higher EC levels, and could be due to salinity. High NaCl concentrations resulted in a significant suppression of *M. javanica* under laboratory and greenhouse conditions[35]. Under greenhouse conditions, infectivity of *M. incognita* was reduced at higher NaCl (EC 5) concentration than at EC

1.5[9].

At EC8, NH_4Cl followed by $(NH_4)_2SO_4$, increased salinity without negatively affecting cucumber growth and yield. Fertilizer components might be lethal directly to nematodes, or might alter pH and/or salinity of the soil harboring nematodes[13; 31; 39].

Diammonium phosphate was best for increasing cucumber growth and yield despite root-knot infection. Symptoms of root-knot nematode infestation appears as stunted growth coupled with severe deficiency symptoms of some nutritional elements, loss of yield and a reduction in product quality[21]. Use of $(NH_4)_2HPO_4$ through its nutritional effect could compensate for damage caused by the RKN. Addition of $(NH_4)_2SO_4$ reduced total number of plant-parasitic nematodes; reduced root galling induced by *M. incognita* on cucumber; increased numbers of free-living nematodes, and resulted in increased fresh and dry weights and length of cucumber plants over the non-treated control[3]. In contrast to calcium salts that had no effect on juveniles of *M. incognita*, ammonium salts were strongly repellent[22].

Furthermore, the application of ammonium fertlizers was found nematicidal on other plant-parasitic nematodes (Walker, 1971; Castro *et al*., 1991; Sudirman and Webster, 1995; Le Saux and Queneherve, 2002). Ammonium ion may affect nematode ability to supply energy for egg-hatching and root invasion and infection (Scott and Martin, 1962; Viglierchio, 1979; Orion *et al*., 1980; Orion *et al.*, 1995).

Our study suggests the limited use of ammonium-containing fertilizers as a relatively safer alternative for chemical pesticides for managing the root-knot nematode effectively in those fields infested with the nematodes. Combination of ammonium amendments with solarization or a bioagent may improve their efficacy in pest management [27]. Activity against *M. incognita* was improved when soil solarization was used in conjunction with amendments such as ammonium phosphate or composted chicken litter[11]. Therefore, further studies are needed to investigate the effects of $(NH_4)_2HPO_4$ and $(NH_4)_2SO_4$ in a combination with other tactics to manage the root-knot nematode, *M. javanica* on cucumber.

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