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Effects of salt -affected soil ameliorated with gypsum, compost or sulphuric acid on the reproductive parameters of root knot nematode, *Meloidogyne incognita* infecting tomato plants var. castle rock under green house conditions

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Abstract : The effects of increasing salt concentration measured in terms of electrical conductivity on nematode developmental parameters and plant growth response in ameliorated and non ameliorated soil were studied on tomato var. castlerock under greenhouse conditions. The results revealed that in salt-affected soil, infected control plants without ameliorants exhibited significant variations in nematode reproduction and plant growth response. The present results indicate that increasing salt concentration up to EC 2 resulted in significant decrease in number of juveniles in soil, number of egg masses, number of galls associated with mild increase in plant growth parameters in control without ameliorants. With increasing salinity up to EC 3, data indicated significant increase in juvenile's number in soil, and significant increase in plant growth in control without ameliorants. On the other hand, tomato plants grown on salt- affected non infected soil without ameliorants exhibited significant decrease in plant height which was correlated with the degree of salinity. The results also show that application of gypsum, compost, and sulphuric acid produced variable effects on nematode development and plant growth response. The most remarkable percentage decrease in number of juveniles in soil associated with highest increase in plant growth was found by application of compost at low salinity level (EC1). Although the application of gypsum and sulphuric acid as soil ameliorants decreased the nematode growth parameters, there were less remarkable effects on plant growth parameters as compared to the infected control without ameliorants. On the other hand, the application of soil ameliorants gypsum, compost or sulphuric acid on salt-affected, non infected plants revealed that the highest plant length was observed by application of gypsum at medium salinity level (EC2).

In conclusion, compost improves tomato plant growth infected by *Meloidogyne incognita* at low salinity levels. Moreover, gypsum enhances non infected tomato plants at medium salinity levels. Generally it could be assumed that the influence of salt concentration on the development and reproduction of nematode depends on nematode species, plant variety, and soil electrical conductivity

Key words: soil salinity, ameliorants, gypsum, compost, sulphuric acid, root knot nematode, tomato plants.

Introduction

In Egypt the nematode *Meloidogyne* spp. is a serious pathogen infecting tomato plants (Ibrahim [1]) in newly reclaimed areas, this problem appears to have economic ramifications. These regions are characterized by high evapotranspiration rates, due to high temperature, and low relative humidity, which may result in tremendous accumulation of salts in root zones. The ground water in the newly reclaimed sandy soils of the desert inflates the problem by gradually increasing the soil salinity year after year. Such increase in soil salinity adversely affects the growth of the cultivated field or crops (Bernstein,[2]). It has also been found to affect the soil nematode. (Jairajpui *et al*., [3], Edongali and Ferris ,[4],Youssef *et al*. ,[5] , and Karajeh and Al –Naser [6]).

In Sahl El-Hossynia soil degradation caused by salinizations and sodication is rampant and poses a major threat and concern in Egypt. Saline (EC > 4 dSm-1), or salt- affected soil is a major environmental issue, as it limits plant growth and development, causing productivity losses (Qadir *et al*., [7]). Salt- affected soils are characterized by excessively high levels of water- soluble salts, including sodium chloride (NaCl), sodium sulfate (Na2SO4), calcium chloride (CaCl2) and magnesium chloride (MgCl2), among others. NaCl is a major salt contaminant in the soil. It has a small molecule size and when oxidized by water, produces sodium ions (Na^{+1}) and chloride ions (Cl^{-1}), which are easily absorbed by the root cells of higher plants and transferred to the whole plant using xylem uploading channels. It also causes ionic and osmotic stresses at the cellular level of higher plants, especially in susceptible species (Tester and Davenport[8], Mansour and Salama [9], Chinnusamy *et al*. [10], Rodriguez-Navarro and Rubio,[11]).

The influence of salinity on nematode infectivity is unclear. Excessive amounts of Na^{+1}, Cl^{-1} might have specific toxic effects on nematode development. Egg hatching of *M.javanica* decreased as the concentration of the electrolytes increased in the solution media (Dropkin ,Martin& Johnson ,[12] , Lal and Yodav,[13].) A positive correlation between citrus nematode populations and chloride content of soil water was observed by Kirkpatric &Van Gundy [14], Mashela *et al*.,[15]. Different effects, increasing or decreasing nematode hatching, developing and reproduction have been observed with different mineral or salt concentrations on various nematode species, (Khan, and Khan, [16], and Sweelam[17])

Vermak *et al*.,[18], reported a negative correlation between *M. incognita* galling, final population and increasing saline conductivity. El-Sayed and Susan[19] studied the effects of different water resources on infectivity, development and reproduction of *M. javanica* and *Rotylenchulus reniformis*. They came to the conclusion that, root – knot nematode development and reproduction was higher with irrigation by ground water. However, reniform nematode development and reproduction was much higher when Nile water was used rather than ground water. There are many procedures that can be used to improve salt- affected land such as water leaching, chemical, and phytoremediation (Qadir *et al* [7] , Feizi *et al* [20]).

The remediation of saline soil using chelating agents such as gypsum $CaSO_4.H_2O$ (Chaudhary,[21]) and organic matter (Tegada [22]) (such as farmyard manure , green manure ,organic amendment) is a fruitful topic of investigation and can be applied worldwide, being low cost , effective and simple to implement (Makoi and verpelancke [23]). The application of gypsum and /or farmyard manure as remediation of sustainable land usage and crop productivity leads to enhanced plant growth and development. (Wong *et al* [24] Cha-um, Suriyan and Chalermpol,[25]).

In Egypt improving salt-affected soil is considered an important part in the agricultural security program. Management of salt- affected soil requires a combination of agronomic practices depending on chemical amendments, water quality and local conditions including climate as well as crop economic policy. (Ismail *et al*., [26]).

The objectives of this study are: to investigate the response of tomato *Lycopersicon lycopersicum* var Castleroc to various levels of soil salinity. 2) To determine the effects of the interaction of *Meliodogyne incognita* and variable levels of salt on nematode development, reproduction, and tomato plant growth response. 3) Remediate salt–affected soil by using either gypsum, compost, or sulphuric acid and their effects on nematode development and plant growth response.

Materials and Methods

The effects of salt- affected soil induced stress on the nematode development, reproduction, and remediation of salt- affected soil by using soil ameliorants were studied under green house conditions, at Agriculture research centre, Cairo, Egypt. Tomato seeds var cacstlerock were germinated and grown in vermiculate at 30 $^{+}_{-}$ 5^{0c}. 120 pots each containing one Kg of dried salt- affected soil with either E.C 1, E.C 2or E.C 3 were prepared. Soils were taken from the farm at Sahl El-Hossynia, Agric. Res. Station El-Sharkia-Governorate, Egypt.

At the beginning of the experiment soil samples were collected and subjected to the analysis of some soil chemical properties, as described by Cottenie *et al.* [27]).The physical and chemical properties of soils are shown in Table (1).

Table 1: Some physical and chemical characteristic of studied soil.

No. of soil	Soil depth (cm)	pH	E.C dsm^{-1}	OM	Gypsum %	Sand%	Silt%	Clay%	texture	CEC Meq.100 g soil	ESP%
1	0-30	9.28	7.1	0.83	1.7	13.6	33.8	52.6	clay	46.1	31.2
2	0-30	8.86	10.01	0.84	1.9	14.2	38.4	47.5	clay	42.4	38.5
3	0-30	8.14	12.02	2.08	0.4	16.5	29.5	54.0	clay	41.3	41.3

The three selected electrical conductivities EC levels were, EC 1= 7.1dSm^{-1}, EC 2=10.01dS m^{-1}, and EC 3= 12.01 dSm^{-1}, according to (Page *et al.*, [28]). 10g gypsum, or 10g Compost, or 6ml sulphuric acid were added to each pot in every group of soil remediate to supply the optimum concentration of approximately half the recommended dose for each remediate. An additional zero remediates control treatments were included.

Table (2): Some characteristics of compost applied in the Experiment

Experimental year	pH 1:10	E.C dSm^{-1}	OM %	Total N %	Total P%	Total K%	Available P%	C/N ratio	Ɵw %
2014	8.02	3.19	66.7	1.8	0.65	0.72	82.31	21.5	19.4

Each treatment for each EC level with or without nematode inoculation was replicated five times. After thirty five days from tomato seeds' germination, two tomato plants were individually transplanted to each pot. The pots were irrigated using tap water to maintain the soil moisture content at 70% of field capacity. Seven days later, the cultivated pots were divided into two groups. Each pot of the first group was inoculated with 2000 freshly hatched second stage juveniles of root–knot nematode *Meloidogyne incognita* in holes around the plant roots.

The second group was kept without nematode inoculation to serve as control treatments. The experiment was designed in a completely randomized block design. Sixty days after inoculation, the experiment was terminated and plant heights, shoot fresh weights, were measured. Number of galls, and number of egg masses were determined. Final nematode populations were measured according to (Barker [29]). The data was analyzed statistically as mean effects for each parameter of nematode reproduction or plant growth studied under different soil salt-affected soils according to Snedecor, and Cochran [30]).

Results

Table 3 (A,B,C) illustrates the effects of increasing salt concentration in the soil measured in terms of electrical conductivity (EC) on nematode development, reproductive parameters and plant growth response. In control infected without ameliorant treatments the results in (Table 3-A, 3-B, 3-C.) revealed significant variations in nematode reproductive parameters; number of juveniles in soil, number of galls, and number of egg masses. The number of juveniles in soil was found to be 3750, 2750, 4750, at (E.C 1, EC 2 and EC 3) respectively. The number of galls was found to be 110, 45, and 110 at (EC 1, EC 2, and EC3) respectively. While the number of egg masses was 95, 33, and 85 (EC1, EC2, and EC3) respectively. The rate of nematode build up was 1.9, 1.3, and 2.4 at EC1, EC 2, and EC3 respectively. Meanwhile, the average plant height and shoot weight in this experiment were (35cm-6 g), (40cm -7g) and (45 cm – 9 g) at EC 1, EC2 and EC 3 respectively.

On the other hand, tomato plants grown on salt-affected, non- infected soil without ameliorants exhibited significant decrease in plant height, which was correlated with the degree of salinity. The plant heights were 32.5 cm, 21.5 cm, and 18 cm at EC 1, EC 2 and EC3 respectively. (Table 3- A, B, and C). The results in Table, 3-A, 3-B, and 3-C also show that application of different chemical and organic soil amendments such as, gypsum, compost, and sulphuric acid produced variable effects on nematode development, reproduction, rate of build up parameters and plant growth responses.

The most remarkable percentage decrease in number of juveniles in soil, number of galls, number of egg masses and nematode rate build up associated with the highest increase in plant lengths and shoot weights was found by application of compost at EC1.

They were 61.6%, 40.9%, 57.8% and 0.7 % respectively as compared to control infected plants without ameliorants. The plant height and shoot weight was 45 cm – 10 g respectively. Although the application of gypsum and suphluric acid as soil ameliorants decreased the nematode growth parameters, there were less remarkable effects on plant growth parameters as compared to the infected control plants without ameliorants. Table 3 -A.

With increasing soil salinity up to EC2, application of gypsum or compost or sulphuric acid showed significant increase in nematode population associated with slightly significant effects on plant growth, except in the case of compost, in which the plant growth was more prominent. The plant growth was (44cm- 10g) compared to the infected control plants without ameliorants. Table 3-B

Further increase in soil salinity up to EC 3, the nematode reproductive parameters revealed a similar pattern to that observed at EC 1. However, significant decrease in plant growth was observed by using compost as ameliorant at this level of salinity compared to the infected control plants without ameliorants. Table3-C.

On the other hand, the application of soil ameliorants gypsum, compost, or sulphuric acid on salt-affected non infected plants revealed that the highest plant length was observed by application of gypsum at EC 2 compared to the non infected control plants without ameliorants. Table 3-B.

Table 3: Effects of the levels of salinity A,B,C, and ameliorants on tomato plant growth and reproduction of *Meloidogyne incognita*.

Treatment	Salt-affected soil with infected plants											Salt- affected soil with non-infected plants	
	No. of J2 in1kg soil *	% percent-tage change from control	No. of galls in 1g root	% Percen-tage change from control	No. of egg masses in 1 g root	% percentage change from control	Rate of nematode build up **	Plant height Cm.	% percentage change from control	Shoot weight /g	% percentage change from control	Plant height Cm.	% change from control
A- EC1.													
Control	3750a		110.0 a		95.0 a		1.9	35.0c		6.0d		32.5a	
Gypsum	3550b	- 5.3	90.0 b	18.1	77.0 b	-18.0	1.8	33.0d	-5.7	8.0b	+33	32.5a	0.0
Compost	1450d	- 61.6	65.0 d	40.9	40.0d	-57.8	0.7	45.0a	+28.5	10.0a	+66.6	24.5c	-24.6
Sulphuric acid	3000c	- 20.0	75.0 c	31.8	60.0 c	-36.8	1.5	36.0b	+2.8	7.0c	+16.6	30.0b	-7.6
B- EC2													
Control	2750d		45.0d		33.0c		1.3	40.0c		7.0b		21.5c	
Gypsum	5500a	+ 100.0	65.0b	+ 44.4	35.0b	+ 6.0	2.7	42.0b	+5.0	6.0c	-14.2	35.0a	+62.7
Compost	3350b	+ 21.0	70.0a	+ 55.0	60.0a	+ 81.8	1.7	44.0a	+10.6	10.0a	+42.8	19.0d	-11.6
Sulphuric acid	3000C	+ 9.2	60.0c	+33.0	55.0b	+ 66.6	1.5	40.0c	0.0	6.0c	-14.2	22.0b	+2.3
C- EC 3													
Control	4750a		110.0a		85.0		2.4	45.0a		9.0a		18.0b	
Gypsum	4050b	- 14.7	95.0b	- 18.0	70.0	- 17.0	2.0	4.02b	-6.6	7.0b	-22.2	13.0c	-27.7
Compost	2750d	- 42.1	60.0d	- 45.0	50.0	-44.0	1.4	42.0b	-6.6	7.0d	-22.2	13.0c	-27.7
Sulphuric acid	3250C	-31.5	70.0c	- 36.0	55.0	- 33.0	1.6	33.0c	-6.6	6.0c	-33.3	20.0a	+11.11

*The same letter non significant at .05

**Rate of nematode builds up: pf / pi pf=final nematode population + No. of galls, pi=initial population

A- EC1: Electrical conductivity at level: 7.1DSm^{-1}

B- EC2: Electrical conductivity at level: 10.01DSm^{-1}

C- EC3: Electrical conductivity at level: 12.02DSm^{-1}

Discussion

Under greenhouse conditions, the interaction of *Meloidogyne incognita* and soil salinity was studied to clarify the role of soil salinity on nematode development, reproduction, and the response of tomato plants. The present results Table 3-A, 3-B, 3-C indicate that , increasing salt concentration up to EC 2 resulted in significant decrease in number of juveniles in soil, number of egg masses, number of galls and consequently nematode rate of build up associated with mild increase in plant growth parameters in control without ameliorants (Table 3- B). With increasing salinity up to EC 3, data indicated significant increase in juveniles' number in soil, and significant increase in plant growth in control without ameliorants Table 3- C. The opposite was observed in the growth response of salt-affected soil of non infected tomato plants. With increasing salinity the plant height significantly decreased in control without ameliorants Table 3-A, 3-B, 3-C.

These results are in agreement with the findings of Ezorug *et al*., [31]. In their study; The influence of salt concentration on development of *M. incognita*, they reported that tomato plant growth and yield was reduced very little in the absence of nematodes at salt concentrations at or below EC 2.5. However, with nematode infection the plant growth reductions were observed at lower salt concentrations.

Kirkpatrick and VanGundy, [14] found that the development of *Tylenculus semipenetran* increased by increasing salinity until 6.5 momhes / cm. Salinity increased egg production of *T. semipenetrans* by 2-10 folds when Nacl was added in irrigation water every other day (Mashela *et al*., [15]). Other researchers observed reduction in nematode activities as a result of increasing salt concentrations. Sweelam [17], found that gall numbers caused by *Meloidogyne javanica* on tomato roots were reduced by increasing sodium chloride concentrations from 1000 to 4000 ppm.

The effects of soil salinity and *M. javanica* on tomato plants were studied by Maggenti and Hardan [32], they found that with increasing salinity to EC 4, the nematode population level initially dropped to less than one – half. Beyond that salinity level, the population remained essentially constant. Vermak *et al*., [18] as well stated that galling , egg masses, egg/ egg masses and final population in the soil decreased with increasing EC levels from 5.6 – 11.2. Al – Sayed *et al*., [33] revealed that, increasing salt concentration resulted in significant increase in the number of galls, the number of eggs / g soil as well as the final population. The highest rates of build up were observed at 2000 ppm Nacl (EC 3.16 – 3.21, 3.12 – 3.17) on egg plant and squash and at 1000 ppm on cowpea (EC 1.88 – 2.13).

Generally it could be assumed that influence of salt concentration on the development and reproduction of nematode depend on nematode species, plant variety and soil EC as shown in the previous discussion.

The effects of root knot nematode on control plants grown under salt-affected soil conditions without ameliorants are complex, and plant height measurements alone provide only a partial picture (Maggenti and Hardan [32]). In the present work, in the control without ameliorants at lower salt concentration, the nematodes cause a decline in vegetative growth Table 3– A. With increasing salt concentration up to EC2 mild increase in vegetative growth and plant height was observed Table 3–B. However, recovery of the plant growth at EC 3 was clearly observed Table 3–C. The decrease in vegetative growth and plant height at E.C1and the mild increase at EC2 and the highest increase at EC3 may be explained by the effect of salinity on absorption of nutrients and plant tissue formation.

The salinity at EC1 may have influenced the balance of nutrients causing a decrease in growth and mineral absorption. Concurrently, the salt concentration at EC2 was not high enough to cause a notable increase in the osmotic pressure of the soil solution.

At EC3 the osmotic pressure of the soil solution was high enough to cause a compensatory increase in the concentration of the cell sap. It has been suggested that plant parasitic nematodes might convey accentuating effects on plant growth under high salinity conditions (Maggenti and Haradan [32]). This view supports the results of this study that plant height and shoot weight were significantly increased at EC3 accompanied by significant increase in nematode population Table 3- C.

Concerning the initial drop in nematode population at EC2 and then the increase in population at the higher level EC3 may reflect some adaptation mechanisms in nematode population in response to salinity induced stress.

Surprisingly, non infected control plants grown on salt-affected soil without ameliorants did not show the same pattern observed in infected tomato plants. There was a negative dose response between the concentration of salinity and plant growth. This result supports our suggestion that the nematode may have positive modifying effects on plant growth.

The interaction of soil ameliorants, such as organic matter and gypsum, have been extensively studied in different concentrations of salinity in non infected economical crops, (Chaudhry,[22], Gahfoor *et al*.,[35], Tegada,[23], Cha-un and Chaleenmpol[26]).

However, interactions of salinity, soil ameliorants and nematode infection on plant growth received little attention in the available literature.

The present study revealed that remediation of low soil salinity by compost, produced significant decrease in nematode reproduction and development. In addition, compost treatment was associated with a remarkable increase in plant growth response as compared to control infected without remediation Table 3- A.

It has been assumed that organic matter may function as binding agents for toxic ions (Zaka *et al*., [33]). Other authors have found that organic matter applications to saline paddy soil is an effective procedure in terms of the physical, chemical and the biological properties of the soil (Wong *et al*., [25]), which can be used to enhance growth and development of the rice crops prior to grain harvesting (Gahfoor *et al*., [34], and Mortaza, [35]). From our previous experience on treated Cowpea plants infected with *Meliodogyne incognita* by using organic matter the results indicated decreased nematode population and increased plant growth response. This was attributed to the fact that the products from decomposing organic matter are directly toxic to nematodes. Also, soil amendments make the important elements in the soil available for plants and thus increase the nutrient uptake, that enhances both plant growth and resistance against nematodes, (El- Gindi *et al*., [36]).

As for using gypsum or sulphuric acid as soil salinity modifiers in infected tomato plants, the present results indicate less remarkable effects on both the nematode reproductive parameters and plant growth parameters, as compared with application of compost at low salinity levels. Conversely, application of gypsum produced remarkable enhancement of plant growth in non infected tomato plants at EC2 level Table 3-B. By increasing the level of salinity EC3, the present results reveal significant decrease in nematode reproductive parameters by application of compost, followed by sulphuric acid and gypsum associated with significant decreases with plant growth parameters.

The present work clearly demonstrates the beneficial effects of gypsum as soil ameliorant only in the non infected tomato plants at moderate salinity levels. The beneficial effects of gypsum could be attributed to its flocculating properties, i.e. soil particles come together and form natural aggregates that improve soil porosity (Davidson [37]). In addition, the effects of gypsum as pH of salt-affected soil could not be neglected.

Conclusion

Finally, under the same conditions of this study and from the previous discussion it can be concluded that the effects of soil ameliorants such as compost could be used to improve the growth of tomato plants infected by *Meloidogyne incognita* in low soil salinity. In addition gypsum may be used as soil ameliorants in non infected tomato plants at medium salinity levels. However, more investigation is still required to study the effects of different nematode species as well as plant varieties in reclaimed fields.

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