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Impact of Organic and Inorganic Fertilizers on Nematode Reproduction and Biochemical Alterations on Tomato

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Abstract

The organic amendments, compost, neem and poultry as well as inorganic fertilizer, N P K and nematicide Nemacur 10% G applied singly at two different doses were effective in reducing *M. incognita* number of galls, nematode reproduction and fecundity. Also, they ameliorated growth criteria of treated tomato plants. The effectiveness seemed to be material origin and concentration dependent. Neem, compost 1, 3 at higher doses (5 g/pot) gave the best results. Yet, achieved results were less than those of nemacur 10% G which overmatched all the organic and inorganic fertilizers. Nematode infection reduced total soluble sugars in roots but the opposite was the case in all treatments. Nematode infection supported root contents of amino acids, total phenols and tannins but they were diminished as a result of almost all treatments. Total soluble sugars and total carbohydrates in shoots decreased as a result of nematode infection but they were regained only by application of inorganic fertilizer. Total amino acids increased in shoots of infected plants and more increase was observed in almost all treatments. Nematode infection impaired tomato uptake of N P K; organic and inorganic fertilizers provoked plants up take, however nemacur improved plants up take of nitrogen only.

Keywords: *Meloidogyne incognita*, organic fertilizers, inorganic fertilizers, biochemical alterations

Introduction

Application of organic matter to the soil is known to have beneficial effects on soil nutrition, soil physical conditions, soil biological activities and crop performance (Efthimiadou *et al*., 2010). In addition, these materials have also been investigated as an alternative method of nematode management (Akhtar and Mahmood, 1996). Plants grown in soil that is high in organic matter often are less damaged by nematodes than plants grown in soil with less organic matter content (Al-Rehiayani, 2001; Efthimiadou *et al*., 2009). Any kind of organic soil amendment including compost can both improve tolerance of plant to nematodes and also reduce nematode populations. However, they cannot eliminate a severe nematode infestation. They are better suited to keep nematode population relatively low than reducing high ones (Crow and Dunn, 1994). Reductions in population densities of phytoparasitic nematodes in response to application of animal-culture manure especially poultry droppings and their positive effects on host growth have been reported in many studies (Ahmad and Siddiqui, 2009; Farahat *et al.,* 1999). Neem, *Azadirachta indica* as a potanical material has been reported to posses nematotoxic activity due the content of limonoids and azadrachtin (Strilling, 1991). Addition of neem to the soil depressed phytonematodes populations (Anjum *et al.,* 1996). There has been considerable progress in the use of compost as soil amendment for the control of plant parasitic nematodes in infested fields (Akhtar and Malik, 2000; McSorley *et al.,* 1999; Zhang and Zhang, 2009).

The research approach of using inorganic fertilizers to diminish nematodes and maximize the benefits of fertilizer started in 1955, when Oteifa reported that ammonia decreased the counts of *M. incognita* females and eggmasses produced on infected lima beans. Many reports have been published dealing with that field of study (Akhtar *et al.,* 1998; Kheir *et al.,* 2009; Oteifa, 1955; Sinha and Neog, 2003).

Chemical alterations in plant tissues due to root-knot nematode infection had been extensively studied. Such alterations included total and reduced sugars, total carbohydrates, total, soluble and insoluble protein and amino acids, phenols, fatty acids as well as minerals in different host plants (Alam *et al.,* 1976; Farahat *et al.,* 2007; Jain and Kant, 1991; Nasr *et al.,* 1980; Parveen *et al.,* 2006; Sharma and Trivedi, 1997; Shuying and Zhixin, 1999).

The aim of current study was to investigate the impact of organic and inorganic fertilizers on nematode reproduction and its effects on the biochemical constitutes on tomato.

Materials and methods

One month old tomato (*Lycopersicon esculentum*) seedlings cv. 'GS' with uniform size were transplanted singly

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in 15 cm clay pots filled with virgin sandy soil. One week after transplantation, the following materials were applied at two doses as illustrated in Tab. 1. Materials were incorporated with the pot soil around the plant. Each treatment was replicated four times. One week later, each pot was inoculated with 3000 freshly hatched juveniles (J_2) of *Meloidogyne incognita* by pouring the nematode water suspension into 4 holes around root system and immediately covered with sand. Control pots were inoculated and kept free from any material as well as non inoculated healthy plants. All treatments were arranged on a greenhouse clean bench at 32±5°C and watered daily for 45 days. Then, plants were lifted out and data on plant growth were recorded and nematode population in soil and roots was counted. Shoots and roots of healthy and infected plants from the treatments of the higher doses (5 g/pot of organic matter, 0.2 of the nematicide) were harvested for chemical analysis.

Total soluble sugars, total carbohydrates and total amino acids were determined by colorimetric methods (Chow and Landhausser, 2004; Etsushiro *et al.,* 1981). Total phenol content was determined by the Folin-Ciocalteu method described by Meda *et al.* (2005) and tannins using vanillin hydrochloric acid method according to Burns (1971). Nitrogen was determined according to the method described by A.O.A.C. (1995), phosphorus according to El-Merzabani *et al.* (1997) and Potassium using Advanced Microwave Digestion system, ETHOS1 (ICP Spectrometer (iCAP 6000 Series; Thermo Scientific).

Tab. 1. The organic and inorganic fertilizers treatments

Treatments	Material	Dose (g/pot)
1	Nemacur10%G	0.1
2	Nemacur10%G	0.2
3	Compost 1	2.5
4	Compost 1	5.0
5	Compost 2	2.5
6	Compost 2	5.0
7	Compost 3	2.5
8	Compost 3	5.0
9	Neem	2.5
10	Neem	5.0
11	Poultry	2.5
12	Poultry	5.0
13	A three®	1.0
14	A three®	2.0
15	N P K*	0.3:0.1:0.3
16	N P K**	0.6:0.2:0.6
17	untreated inoculated	
18	untreated healthy	

* 1/2 dose, ** recommended dose Compost 1 (Rice straw + Okara + Rock phosphate, C/N ratio 33.94), Compost 2 (Rice straw + Okara + Rock phosphate + Composite inoculum C/N ratio 32.13), compost 3 (Rice straw + Okara+ Rock phosphate + Composite inoculum + buffalo manure C/N ratio 32.73). The three types of compost were produced (Rashad *et al.*, 2010) and obtained from Microbiology Department, Faculty of Agriculture, Cairo University

Reducing sugars and total fatty acids were determined according to Holme and Peck (1983) and Farag *et al.* (1986) respectively.

Statistical analysis was carried out using Analysis of Variance (ANOVA) of MSTAT-C statistical package (Michigan State University) (MSTAT-C 1989).

Result and discussion

Nematode development and reproduction

Data presented in Tab. 2 reveal that all tested organic amendments applied at the two doses (2.5, 5.0 g/pot) significantly impaired gall formation, juveniles recovered from the soil, the numbers of the developmental stages, eggmasses, size of nematode population, nematode build up and egg production as compared with the untreated check with few exceptions. With regard to the treatments concentration, differences in nematode suppression were distinguishable. Although the lower dose of compost 3 and the higher one of compost 2 increased significantly the number of eggmasses/g root, they significantly decreased nematode fecundity (eggs/eggmass). In contrast, the lower dose of compost 1 and the higher one of poultry significantly increased egg production. Differences in nematode reductions were obvious among treatments and/or doses, the higher the dose the higher the reduction in nematode numbers except for poultry droppings. Neem, compost 1 and compost 3 at higher doses, in that order, gave the best results.

Concerning the inorganic fertilizer, N P K, treatments behaved differently. It is interesting to notice that the commercial product (A three®) was more effective in suppressing the nematode developmental stages, eggmasses and gall formation than the prepared N P K. The lower dose of the compound N P K (0.3:0.1:0.3 g/pot) significantly increased nematode criteria and poorly enhanced nematode reproduction.

The utmost reductions in over all values of nematode criteria were achieved by Nemacur 10% G at the two doses. The nematicide protects the roots from nematode invasion which resulted in sharp reduction in the number of galls, eggmasses, and fecundity and smashing the nematode build up.

The present results emphasized that all organic and inorganic fertilizers exhibited potential activity against the root-knot nematode and improved growth criteria of tomato irrespective of their origin or concentration. All the tested materials including N P K significantly suppressed root-galling, the number of eggmasses, egg production and subsequently the final population. However, the botanical material, neem, seemed to have higher toxic action much more than animal culture, compost and inorganic fertilizer but not as much as nemacur 10% G which overwhelmed all other treatments. Many reports in literature emphasized the role of neem in controlling plant-parasitic nematodes (Farahat *et al.,* 2008; Zaki and Bahati, 1989).

Tab. 2. Reproduction of *M. incognita* as influenced by organic and inorganic fertilizers

Treatments	Dose/pot (g)	Galls/root	Galls/g root	Embedded stages/root	Egg masses/root	Soil population	Final Population	% Reduction	Eggs/ egg mass	Pf/Pi
Nemacur	0.1	12^{p}	1^{j}	5^{o}	12^{o}	0^{p}	17	99.9	130^{h}	0.01
Nemacur	0.2	6^{p}	1^{j}	0^{o}	6^{o}	0^{p}	6	100.0	130^{h}	0.00
Compost 1	2.5	1581^{b}	99^{c}	1125^{c}	924^{g}	4248^{h}	6297	58.0	210^{d}	2.10
Compost 1	5.0	770^{k}	58^{fg}	427^{l}	479^{l}	1563^{n}	2469	83.5	210^{d}	0.82
Compost 2	2.5	1093^{g}	67^{ef}	798^{i}	701^{i}	5416^{f}	6915	53.8	154^{g}	2.30
Compost 2	5.0	1495^{c}	86^{d}	715^{j}	1352^{b}	3910^{j}	5977	60.1	193^{e}	1.99
Compost 3	2.5	1452^{d}	81^{d}	1518^{b}	1210^{c}	1960^{l}	4688	68.7	96^{j}	1.56
Compost 3	5.0	349^{o}	19^{i}	174^{n}	601^{k}	2115^{k}	2890	80.7	113^{i}	0.96
Neem	2.5	1007^{h}	59^{fg}	1105^{d}	614^{k}	1880^{m}	3599	76.0	170^{f}	1.20
Neem	5.0	480^{m}	32^{h}	818^{h}	250^{n}	980^{o}	2048	86.3	163^{fg}	0.68
Poultry	2.5	1253^{e}	70^{e}	635^{k}	1079^{e}	5060^{g}	6774	54.8	192^{e}	2.26
Poultry	5.0	976^{i}	56^{g}	1088^{e}	768^{h}	12250^{b}	14106	5.8	300^{a}	4.70
A three®	1.0	953^{j}	63^{efg}	1073^{f}	1004^{f}	6900^{e}	8977	40.1	197^{e}	2.99
A three®	2.0	375^{n}	27^{hi}	288^{m}	344^{m}	4230^{i}	4862	67.5	310^{a}	1.62
N P K	1/2dose	1844^{a}	111^{b}	1972^{a}	1850^{a}	11500^{c}	15322	-2.3	162^{fg}	5.11
N P K	rec. dose	573^{l}	31^{h}	722^{j}	639^{j}	7130^{d}	8491	43.3	276^{b}	2.83
Control		1198^{f}	272^{a}	1045^{g}	1123^{d}	12810^{a}	14978	-	218^{d}	4.99

In each column, means followed by the same letter (s) are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

The nematicidal activity of neem is due mainly to its content of limonoids and azadrachtin (Strilling, 1991).

Plant growth response

Improvement in plant growth parameters in terms of shoot and root lengths and weights was variable and proportional with the organic and inorganic single treatments concentration Tab. 3. The plants reacted differently according to specific material and an outstanding amelioration was more pronounced in shoot and root fresh weights. There were significant differences between (but not within) many treatments and the control. Increasing nemacur 10% G dose increased significantly root criteria but not shoot ones. Some phytotoxicity were observed on plants received the higher nematicide dose. Doubling the concentration of organic and inorganic fertilizers significantly increased shoot length, dry weight, root length within treatments in many cases. N P K double dose in-

Tab. 3. Growth response of tomato seedlings infected with *M. incognita* as influenced by organic and inorganic fertilizers

Treatments	Dose/pot (g)	Growth criteria							
		Shoot					Root		
		Length (cm)	Fresh Weight (g)	% increase	Dry Weight (g)	% increase	Length (cm)	Fresh Weight (g)	% increase
Nemacur	0.1	30.3^{a-d}	14.3^{dh}	225	2.7^{fg}	80	26.5^{ef}	4.0^{fg}	48
Nemacur	0.2	24.0^{f-h}	12.5^{jk}	184	2.9^{d-f}	93	32.0^{b-e}	4.4^{e-g}	63
Compost 1	2.5	27.0^{c-f}	13.2^{i-k}	200	2.8^{ef}	87	30.0^{b-e}	6.0^{d-f}	122
Compost 1	5.0	26.3^{d-f}	15.9^{e-h}	261	3.0^{cd}	100	34.5^{b-e}	8.4^{bc}	211
Compost 2	2.5	29.7^{a-e}	16.3^{b-e}	270	2.3^{h}	53	29.7^{b-e}	6.3^{c-e}	133
Compost 2	5.0	32.5^{ab}	17.4^{a-c}	295	3.1^{cd}	107	31.5^{b-e}	11.3^{a}	319
Compost 3	2.5	27.7^{cf}	17.9^{ab}	307	3.4^{ab}	127	29.0^{c-e}	4.4^{e-g}	63
Compost 3	5.0	31.0^{a-c}	18.0^{a}	309	3.5^{ab}	133	31.7^{b-e}	8.8^{b}	226
Neem	2.5	26.0^{e-g}	15.2^{e-j}	245	3.0^{cd}	100	28.0^{d-f}	5.0^{ef}	85
Neem	5.0	33.7^{a}	17.2^{a-e}	291	3.3^{bc}	120	31.5^{b-e}	9.7^{ab}	259
Poultry	2.5	30.7^{a-c}	17.0^{a-f}	286	3.3^{bc}	120	36.0^{a-c}	7.6^{b-d}	181
Poultry	5.0	33.0^{ab}	17.3^{a-e}	293	3.4^{ab}	127	40.0^{a}	8.4^{bc}	211
A three®	1.0	26.5^{d-e}	14.1^{f-j}	220	2.9^{d-f}	93	29.5^{b-e}	6.3^{c-e}	133
A three®	2.0	29.0^{b-e}	15.1^{d-i}	243	2.9^{d-f}	93	37.0^{ab}	8.6^{b}	219
N P K	1/2 dose	30.0^{a-e}	16.6^{b-f}	227	3.1^{cd}	107	31.5^{b-e}	7.9^{b-d}	193
N P K	rec. dose	32.7^{ab}	18.2^{a}	314	3.6^{a}	140	33.0^{a-e}	11.6^{a}	330
Control		20.7^{h}	4.4 l		1.5^{c-k}		21.0^{f}	2.7^{g}	

In each column, means followed by the same letter (s) are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test creased significantly shoot and root fresh weights but not their lengths. In this regard, compost 2, compost 3, compound N P K and poultry accomplished the best results.

The present results showed that, compost 3 achieved very good results in both controlling the root-knot nematode and ameliorating the infected plants. These make it suitable for organic farming systems to keep nematode populations always under the economic threshold and improve plant performance if it used regularly.

Results of treatments included compost were significantly different from those of poultry or N P K and achieved overgrowth of the infected plants. The efficacy of compost seemed to depend upon the components of which it was produced. This may explain the differences observed in the present work between the three tested types of compost. The present results are in harmony with those of Moselhy (2009) who reported that compost significantly reduced the formation of galls on sunflower roots, the developmental stages and the final population by 84%. Many other reports proved that compost application improved growth of infected plants and diminished nematode populations (Cayuela, 2008; Lerog *et al.,* 2007). The beneficial effects of compost on treated plants rather than its role in controlling nematodes are outlined by many others as it improves soil structure, porosity, increases infiltration and permeability of heavy soils, improves water holding capacity, supplies significant quantities of organic matter, improve cation exchange capacity, improves and stabilizes soil pH, provides humus, vitamins, hormones and plant enzymes which are not supplied by chemical fertilizers (Ahmad *et al*., 2009; Evanylo *et al*., 2008; Field Guide to Compost Use, 2001; Rashad *et al*., 2010).

The dual effect of N P K in improving plant growth and suppressing nematode populations is documented (Akhtar and Mahmood, 1996).

Biochemical alterations in tomato plants as influenced by Meloidogyne incognita and application of organic and inorganic fertilizers

Biochemical alterations in tomato roots and shoots as a result of the root-knot nematode infection and application of organic and inorganic fertilizers are illustrated in Fig. 1

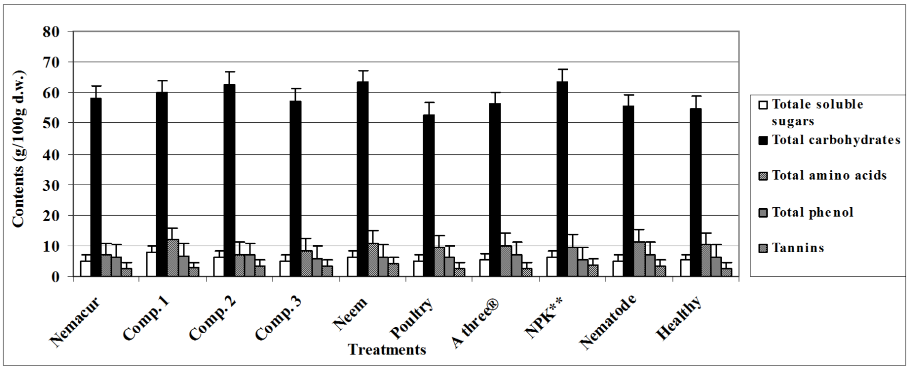

Fig. 1. Biochemical contents (g/100 g d.w.) alterations in tomato roots as influenced by *M. incognita* infection and the application of organic and inorganic fertilizers.

Treatments dose (g/pot): Nemacur (10% G): 0.2; Comp. (Compost) 1, 2, 3: 5 g/pot; Neem (dry leaves): 5 g/pot; Poultry (droppings): 5 g/pot; A three® (three types of compost): 2 g/pot; N P K** (recommended dose); Nematode: Nematode only; Healthy: Untreated healthy

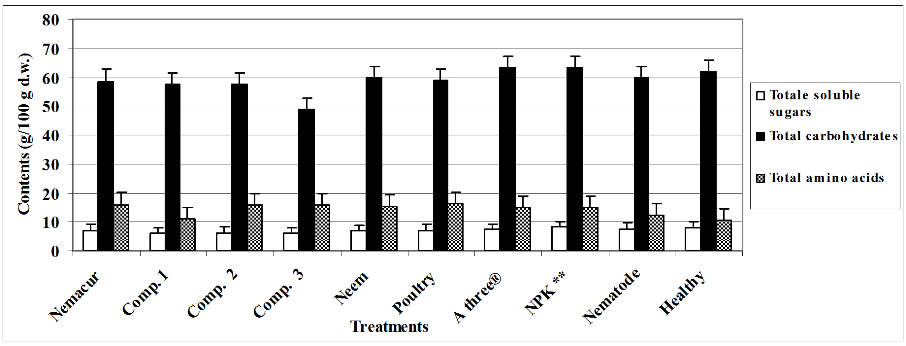

Fig. 2. Alterations in tomato shoots contents (g/100 g d.w.) of soluble sugars, carbohydrates and amino acids as directed by *M. incognita* infection and the application of organic and inorganic fertilizers.

Treatments dose (g/pot): Nemacur (10%G): 0.2; Comp. (Compost) 1, 2, 3: 5 g/pot; Neem (dry leaves): 5 g/pot; Poultry (droppings): 5 g/pot; A three® (three types of compost): 2 g/pot; N P K** (recommended dose); Nematode: Nematode only; Healthy: Untreated healthy and 2. In roots, nematode infection reduced the total soluble sugars by 6.7%. However, all treatments (except poultry droppings) increased roots content of soluble sugars as compared to infected untreated tomato plants. Moreover, compost 1, 2, neem, A three® and N P K increased total soluble sugars over the untreated healthy plants. Almost similar results were observed with total carbohydrates.

In contrast, nematode infection boosted roots contents of amino acids, total phenols and tannins. Almost all treatments (except compost 1, and neem) diminished roots content of total amino acids. Nemacur 10% G achieved the greatest diminution, 31.79, 38.24% from those recorded in untreated healthy and untreated infected plants, respectively.

Also, all treatments brought phenol contents back to be almost near to those in the untreated healthy plants except for compost 2 and the A three® which were very close to those recorded in infected untreated plants. Tannins rose in tomato roots after nematode infection; the majority of treatments accomplished the same results.

Neem treatment raised content of tannins by approximately 1.5 times over those in the healthy plants, however, nemacur, poultry droppings and A three® failed to increase tannins over the untreated healthy roots.

In shoots (Fig. 2), nematode infection decreased the total soluble sugars and total carbohydrates. All treatments failed to reimburse soluble sugars and carbohydrates except for the prepared N P K and A three®, in that order. Nematode infection increased shoots total amino acids content by 18.63%. All treatment (except compost 1) also increased total amino acids in shoots but with higher percentages with preponderancy of poultry droppings followed by nemacur and all compost treatments.

With plants content of N P K, the root-knot nematode infection precluded tomato uptake of the three elements Fig. 3. Treating infected plants with the organic and inorganic fertilizers perked up plants uptake but only few treatments enabled plants to stand with the uninfected healthy ones, A three®, prepared N P K and compost 1 were the best with N P and K, respectively. Generally the prepared N P K gave the over all best results, however nemacur improved plants uptake of nitrogen only.

It was generally postulated that the adverse influence of organic amendments on phytoparasitic nematodes is referred to numerous factors e.g. increasing host resistance to nematode infection and enhancement of growth performance (Courtney and Mullen, 2008), changing physical soil properties like cation exchanges, water retention and soil arrogation (Alam *et al.,* 1977), producing chemicals during degradation like volatile fatty acids, organic acids, phenols, hydrogen sulphide and nitrogenous compounds (Al-Sayed *et al.,* 2007; Kesba and Al-Shalaby, 2008; McBride *et al.,* 2000; Zaki *et al.,* 2004).

Treating infected plants with fertilizers improve the performance of infected plants by enabling them to recompense root losses of soluble sugars and total carbohydrate and brought phenol contents back to be almost near to those in untreated healthy plants, raising tannins content, diminishing root contents of amino acids to be around those in healthy plants. In the above ground parts of the infected plants, almost all treatments improved plants uptake of nitrogen, however, only prepared N P K improved P and compost 1 improved K. Similarly, mineral fertilizers increased shoots content of total sugars and total carbohydrates.

The reduction in soluble, reducing and non reducing sugars, total carbohydrates in different host plants due to nematodes infection was recorded by many research workers (Farahat *et al.,* 2007; Kheir and Abadir, 1982; Parveen *et al.,* 2006; Sharma and Trivedi, 1997; Shuying and Zhixin, 1999). However, an opposed results were recorded by (Alam *et al.,* 1976; Jain and Kant, 1991). Others did not find any pronounced effects of *M. javanica* or *M. incognita* on sugar content (Singh and Chaudhury, 1974).

Concerning N P K levels in plants infected with the root-knot nematodes, Nasr *et al.* (1980) showed that *M. incognita* and *M. javanica* infection increased phosphorous and nitrogen concentrations in bitter almond. Also, *M. incognita* increased nitrogen content of okra (Sharma and Trivedi, 1997). High levels of nitrogen, phospho-

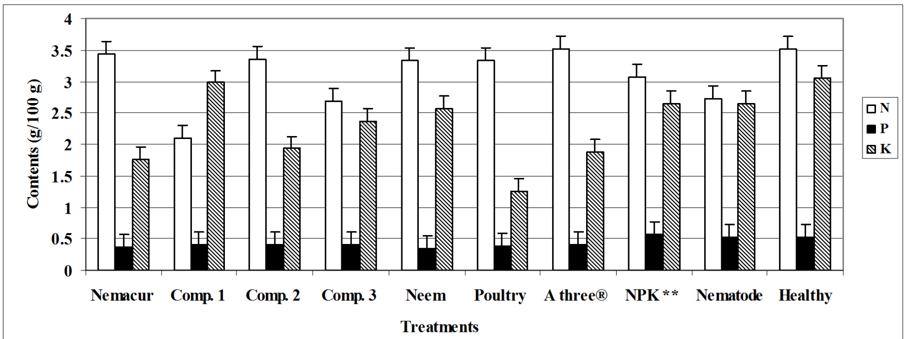

Fig. 3. Tomato shoots contents of N P K affected by *M. incognita* infection and the application of organic and inorganic fertilizers.

Treatments dose (g/pot): Nemacur (10%G): 0.2; Comp. (Compost) 1, 2, 3: 5 g/pot; Neem (dry leaves): 5 g/pot; Poultry (droppings): 5 g/pot; A three® (three types of compost): 2 g/pot; N P K** (recommended dose); Nematode: Nematode only; Healthy: Untreated healthy rous and potassium occurred in root-knot infected than healthy tomato roots (Hunter, 1958; Shafiee and Jenkins, 1963). But the lower contents of minerals in heavily galled tomato roots was illustrated by Oteifa and El-Gindi (1962), when they showed that heavily galled roots can't translocate adequate amounts of nutrients to vegetative organs whereas tomato foliage infected with *M. incognita* have been shown to contain lower concentrations of N P K, sodium, calcium and magnesium.

The phenolic compounds are the best known factors involved in susceptible-resistant response. There is a distinct correlation between the degree of plant resistance and the phenolics present in plant tissues (Abdel-Rahim and El-Beltagi, 2010, 2011; El-Beltagi and Mohamed, 2010; Giebel, 1970; Mohamed *et al.,* 2010). Most phenols occur in plant tissues in bound forms as glycosides of low physiological and chemical activities. Activation requires their decompositions to free phenols (Afify *et al.,* 2011, 2012; El-Beltagi, 2011, El-Beltagi *et al*., 2011a, 2011b, 2011c; Giebel and Wilski, 1970; Kesba and El-Beltagi, 2012; Shallan *et al.*, 2010; Shehab *et al.*, 2010). Nematodes are able to decompose glycosides by secreting β-glycosidases into host tissue (Masseneer, 1964; Wilski and Giebel, 1966).

Tannins are among the chemicals that reduced by nematode infection, it had been shown that tannins are utilized in the formation of necrotic areas around nematodes in resistant host plants, accordingly, tannins increased in such kind of hosts after effect of nematodes (Farahat *et al.*, 2007; McAvoy *et al.*, 1998). However, this is not the case in susceptible hosts like those under the present study.

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