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POMEGRANATE (*Punica granatum* L.) GROWTH AND BİOCHEMİCAL ALTERATİONS İN RESPONSE TO MELOİDOGYNE İNCOGNİTA İNFECTİON, MİNERALS, AND NANO-FERTİLİZERS

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Abstract

The effects of four inoculum levels (500, 1,000, 2,000, or 4,000 second-stage juveniles (J2) per plant) of the root-knot nematode, Meloidogyne incognita, on two pomegranate cultivars ('Manfalouty' and 'Wonderful') were investigated under greenhouse conditions in response to plant inorganic and organic chemical concentrations. Furthermore, the effects of six commercial chemical fertilizers (inorganic fertilizers and nano-fertilizers) on plant growth and nematode reproduction were also studied. Both cultivars recorded the highest gall formation, embedded stages, and final nematode population at the inoculum dosage of 2,000 J2/plant. The highest reproductive rate was achieved with 500 J2/plant, while the lowest rate was observed with 4,000 J2/plant on both cultivars. 'Wonderful', at all M. incognita inoculum levels, was more sensitive to nematode infestation than 'Manfalouty'. 'Wonderful' showed a greater reduction in fresh and dry plant weights than 'Manfalouty' at 2,000 and 4,000 J2/plant. In both cultivars, the concentrations of N, P, and K decreased with increasing nematode inoculum levels. This decline was more pronounced in 'Manfalouty' than in 'Wonderful'. The nano-fertilizers, Hyper Feed®, and Hyper Feed Solo® reduced all nematode parameters but only in 'Wonderful'. Treatment with Hyper Feed® resulted in the highest percentage increases in all plant growth parameters in 'Wonderful'. Total carbohydrate concentration was increased in 'Manfalouty' treated with the nano-fertilizers, especially with Hyper Feed®. Also, concentrations of total phenols and tannins increased in 'Wonderful' when treated with either nano-fertilizer. Generally, both nano-fertilizers showed an increase in plant N levels. We recommend using nano-fertilizers in integrated pest management (IPM) programs on pomegranate where they improved plant growth parameters and reduced nematode multiplication parameters more markedly than inorganic fertilizers.

Keywords: Fertilizers. Inoculum levels. Inorganic fertilizers. Nano-fertilizers. Punica granatum. Root-knot nematode.

1. Introduction

Pomegranate (Punica granatum L.) trees planted in recently reclaimed soils are one of the economically significant fruit crop systems in Egypt (Ismail et al. 2014). Like other important fruit trees, pomegranate is attacked by several species of plant-parasitic nematodes throughout the world, in Jordan

(Hashim 1983), Libya (Siddiqui and Khan 1986), India (Darekar et al. 1989), Pakistan (Nyczepir and Becker 1998), and Egypt (El-Qurashi et al. 2017). The root-knot nematode genus, Meloidogyne, is the most widespread and damaging nematode genus on pomegranates. Most P. granatum varieties and orchards in Pakistan were found to be susceptible to Meloidogyne incognita (Khan and Shaukat 2010). The number of nematodes in the soil and their ability to reproduce on plants were proportional to the plant damage caused (Kesba 2011; Diab et al. 2018). The invasion of roots by infective juveniles increased with increasing nematode density. The threshold density depends on the type of plant, race and species of nematode, and environmental stresses (El-Sherif et al. 2007; Kesba 2011; Diab et al. 2018).

According to several studies, inorganic fertilizers can either directly or indirectly influence how plant-parasitic nematodes develop (Coyne et al. 2004; Irshad et al. 2006). For improved crop production, it is now becoming important to provide external agricultural inputs like fertilizers, particularly the macronutrients NPK, in forms which are also more effective in controlling nematodes than manure or compost (Forge et al. 2005; Hu and Oi 2010). Also, improved plant growth with high tolerance to nematode infestation, combining high yield as well as a marked reduction in nematode reproduction, is increasingly targeted (Kesba and Al-Shalaby 2008; Kheir et al. 2009; Kesba 2010; Farahat et al. 2012). Recently, nano-fertilizers have replaced conventional fertilizers on a wider scale because they are safer for the environment and people, in addition to being less expensive and therefore more profitable for farmers to use (Naderi and Abedi 2012). For example, nano-fertilizers improved cotton plant growth parameters while reducing the number of M. incognita galls and egg masses (El-Sherif et al. 2019).

In the current greenhouse study, we focused on the growth and chemical (inorganic an organic) responses of two pomegranate cultivars ('Manfalouty' and 'Wonderful'), as well as on the reproduction of M. incognita in response to different initial nematode inoculation levels, and the effects of six commercial inorganic and nano-fertilizers to determine whether nematode management and plant growth could be improved by optimizing fertilizer treatments.

2. Material and Methods

Nematode source

From isolates belonging to the Nematology experimental zone, Department of Zoology and Agricultural Nematology, Faculty of Agriculture, Cairo University, the root-knot nematode, M. incognita (Taylor et al. 1955) pure culture was prepared and propagated on tomato cv. Super strain B.

Fertilizers source

Commercial inorganic and nano-fertilizers were obtained from the Egyptian fertilizer suppliers (El Nasr and Bio-Nano Technology Companies, respectively, both in Cairo, Egypt), and their chemical composition are listed in Table 1.

Greenhouse experiments

Plant Preparation

Three-month-old seedlings of uniform size of two pomegranate cultivars, namely 'Manfalouty' and 'Wonderful' were purchased from the Horticulture Research Institute, Agriculture Research Center, Giza, Egypt, and grown individually in clay pots, 20 cm in diameter, filled with (1:1, v/v) steam-sterilized sandy loam soil.

Experiment 1

Five different inoculum rates of M. incognita were used to inoculate the pomegranate cultivars, 'Manfalouty' and 'Wonderful'. Dosages of 0, 500, 1,000, 2,000, and 4,000 J2/plant of M. incognita

inoculum were applied in experiments run in two summer seasons, namely 2021 and 2022. A 2×5 (cultivar and inoculum level) factorial completely randomized design with five replicates of each of the ten treatments was set up in each season. The J2 suspension in water was inoculated onto the roots of each plant suspension by pipetting into four holes drilled into the soil close to each seedling's root system. Uninoculated plants were retained to serve as the control. Otherwise, all seedlings were treated equally and kept in a greenhouse on a bench at a mean temperature of 32±5°C, a mean relative humidity of 75–80%, and an average photon flux density of 1,000–1,100 µmol m−2 s−1.

Table 1. Chemical composition of the commercial fertilizers used in the current study

Content	El Nasr Company			Bio-Nano Technology Company		
	Ammonium Sulfate™	Di-Ammonium Phosphate™	Potassium Sulfate™	Hyper feed®	Hyper Feed Drip®	Hyper Feed Solo®
Nitrogen N %	21	18	-	19	6	10
Phosphor P_2O_5 %	-	46	-	19	40	8
Potassium K_2O %	-	-	50	19	12	36
S %	24	-	18	-	-	-
Fe %	-	-	-	0.48	0.58	0.50
Mg %	-	-	-	0.80	0.80	0.90
Mn %	-	-	-	0.24	0.15	0.25
Zn %	-	-	-	0.35	0.35	0.25
B %	-	-	-	0.05	0.05	0.05
Cu %	-	-	-	0.08	0.08	0.08
Amino Acids %	-	-	-	1.15	1.50	1.50
Algae Extract %	-	-	-	0.52	0.50	0.62
Mo (ppm)	-	-	-	100	100	100
Co (ppm)	-	-	-	100	100	100

-: not detected

Experiment 2

The impact of six commercial inorganic fertilizer products (Table 1) on the development and reproduction of the M. incognita nematode on seedlings of two pomegranate cultivars was evaluated in this experiment. Seedlings of each cultivar were inoculated with 4,000 J2 of M. incognita by pipetting the nematode suspension in water into four holes around the root system, which was then promptly covered with soil. One week after inoculation, seedlings were treated twice (at 2-week intervals) with the fertilizers as a soil drench. All treatments were replicated five times in each of the two seasons (2021 and 2022), and two check treatments, healthy (without nematodes but plus fertilizer) and infested (with nematodes only) were set-up as controls for each cultivar. In the same greenhouse as used in Experiment 1, pots were set up in a completely randomized design, all receiving similar horticultural care apart from fertilizer treatment and nematode inoculation.

Seedlings were lifted at 3 months from inoculation. The plant growth parameters, namely total shoot length (cm), and fresh and dry weights (g) were determined. Following the methodology described by Hooper et al. (2005), the soil was extracted and the gall numbers and egg masses per 5 g stained roots from each plant were counted using a Hawksley counting slide under a binocular microscope. The nematode reproduction rate (Pf/Pi, where Pi = initial population and Pf = final population), and the average number of eggs/egg mass were counted and determined.

Plant chemical analysis

The chemical composition of sub-samples (each 1 g) of the dried whole plant from each treatment was analyzed at the Central Chemistry Laboratory, Faculty of Agriculture Research Park (FARP), Faculty of Agriculture, Cairo University, Giza, Egypt.

The anthrone method, as described by Hedge and Hofreiter (1962), was used to determine total carbohydrate concentration. The Folin-Ciocalteu reagent, as described by Malick and Singh (1980), was used to measure total phenolic concentration. Tannin concentrations were calculated using the Folin-Denis method, as described by Schanderl (1970). Using the Association of Official Analytical Chemists' established procedures (AOAC 1990), the concentrations of N, P, and K were determined.

Statistical analysis

Using the SPSS software program version 23 (SPSS 2015, IBM, Armonk, NY, USA), two-way ANOVA was used to statistically evaluate the obtained data. At a P= 0.05 significance level, Duncan's multiple range tests were used to determine whether there were any significant differences between the analyzed parameters' means by pairwise sample comparisons.

3. Results

Experiment 1: Effect of nematode dosage on nematode multiplication and plant growth parameters

The root-knot nematode, M. incognita, successfully infested, developed, and reproduced on each of the two pomegranate cultivars tested (Table 2). As the inoculum level increased, the numbers of galls and the embedded stages in roots showed a progressive increase with significant differences within or between the two cultivars. In the majority of cases, the multiplication parameters at the higher inoculum dosages varied significantly from those at the lower. The highest numbers of galls, embedded stages, and final population size were recorded in cultivar 'Wonderful' at an inoculum dosage of 2,000 J2/plant; furthermore, the size of the final nematode population was significantly higher in 'Wonderful' than in 'Manfalouty'. A significant negative correlation was observed between the nematode inoculum dose and the rate of nematode multiplication rate (population increase, Pf/Pi), with the highest multiplication rate being associated with the lowest inoculum dosage (Table 2). The highest rate of nematode multiplication was achieved on the 'Wonderful' cultivar There was no significant relationship between inoculum dosage and female fecundity (mean egg number per egg mass) on the 'Manfalouty' cultivar. Meanwhile, the 'Wonderful' cultivar recorded the highest female fecundity with no significant difference between the values associated with different inoculum levels except for a decrease at 2000 J2/plant. It is worth noting that the M. incognita strain was more virulent toward 'Wonderful' than 'Manfalouty' at any given level of nematode inoculum rate, as reflected in nematode fecundity (Table 2).

Table 2. Reproduction of Meloidogyne incognita on pomegranate cultivars as influenced by different inoculum levels.

Inoculum level (J_2/pot)	Nematode counts			Pf/Pi	Eggs/ Egg mass
	Galls/Root	Embedded stages/Root	Final pop. (Emb. + in soil)		
			Manfalouty		
500	513±17.68^{f}	641±22.30^{h}	6401±219.99^{h}	12.8±0.44^{b}	370±10.17^{bc}
1000	747±25.93^{e}	934±32.27^{f}	9334±362.77^{f}	9.3±0.36^{d}	343±18.73^{cd}
2000	1140±35.52^{c}	1424±44.43^{c}	14224±404.98^{c}	7.1±0.71^{e}	340±12.66^{cd}
4000	1021±20.79^{d}	1277±25.98^{d}	12757±299.49^{d}	3.2±0.07^{f}	330±18.04^{d}
			Wonderful		
500	694±16.33^{e}	769±19.52^{g}	7759±159.97^{g}	15.5±0.32^{a}	422±9.08^{a}
1000	1033±23.17^{d}	1152±20.91^{e}	11325±225.07^{e}	11.3±0.23^{c}	392±5.38^{ab}
2000	1520±33.96^{a}	1734±21.17^{a}	17378±195.61^{a}	8.7±0.05^{d}	343±8.40^{cd}
4000	1355±15.94^{b}	1543±17.79^{b}	15219±147.96^{b}	3.8±0.06^{f}	413±7.41^{a}
$F_{7,24}$	198.965	213.206	214.186	269.348	8.742
P	<0.001	<0.001	<0.001	<0.001	<0.001

Means±SD followed by a common letter(s) within a column are not significantly different (P ≤ 0.05) according to ANOVA and Duncan's multiple range test.

With regard to the plant growth parameters, a decrease was observed in all parameters at all inoculum dosage levels when compared with un-inoculated plants (0 J2/plant) except for plant length of 'Manfalouty' at dosage 500 J2/plant and 'Wonderful' at dosage 2,000 J2/plant which coincided with the greatest plant lengths (significantly greater than the 0 J2/plant control) (Fig. 1). The greatest nematode-induced decrease occurred in shoot fresh and dry weight, in response to increasing inoculum levels. It is interesting to note that decreases in fresh (Fig. 2) and dry weights (Fig. 3) in response to nematode inoculation were significant in both cultivars except for the two highest dosages, namely 2,000 and 4,000 J2/plant, with the reductions being more pronounced on 'Wonderful' than in 'Manfalouty' (Figs. 2 and 3).

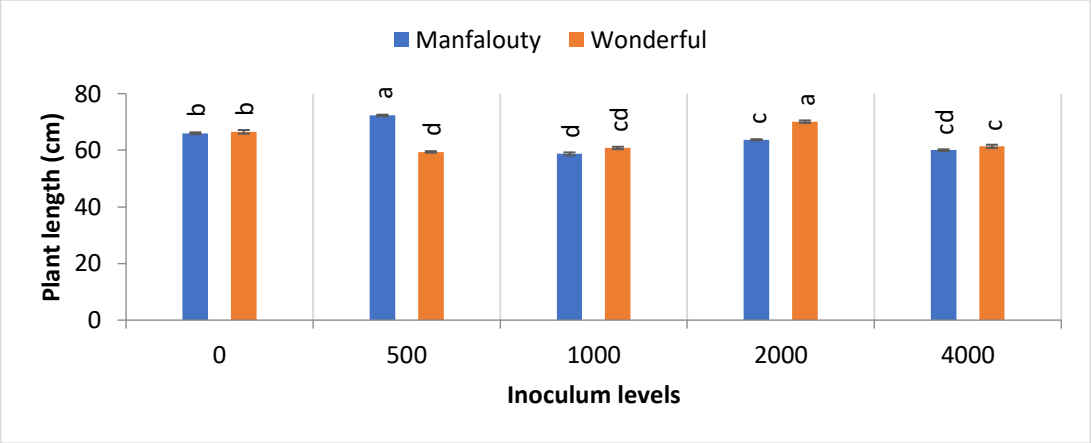

Figure 1. Plant length of two pomegranate cultivars ('Manfalouty' and 'Wonderful') in response to different dosages of Meloidogyne incognita inoculum. Means ± SD followed by a common letter(s) within a column are not significantly different (P ≤ 0.05) according to ANOVA and Duncan's multiple range test.

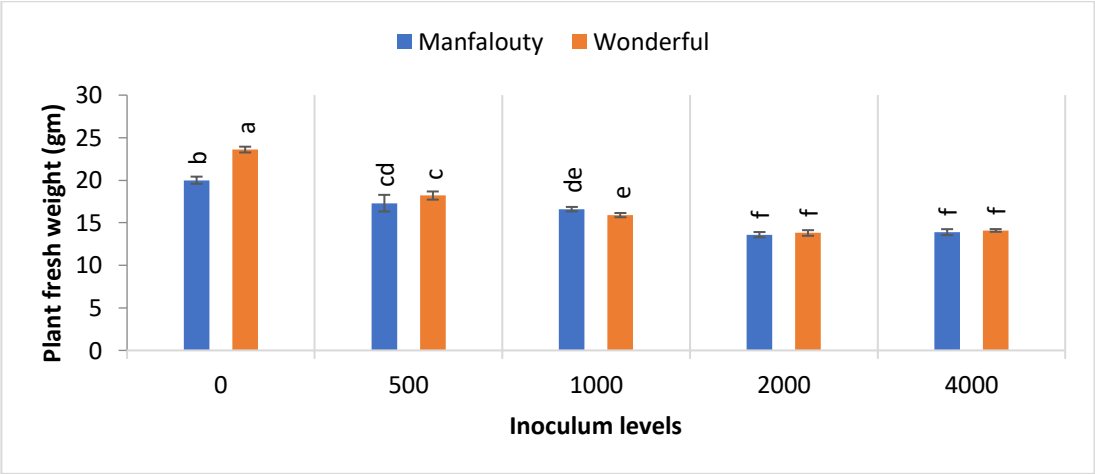

Figure 2. Plant fresh weight of two pomegranate cultivars ('Manfalouty' and 'Wonderful') in response to different dosages of Meloidogyne incognita inoculum. Means ± SD followed by a common letter(s) within a column are not significantly different (P ≤ 0.05) according to ANOVA and Duncan's multiple range test.

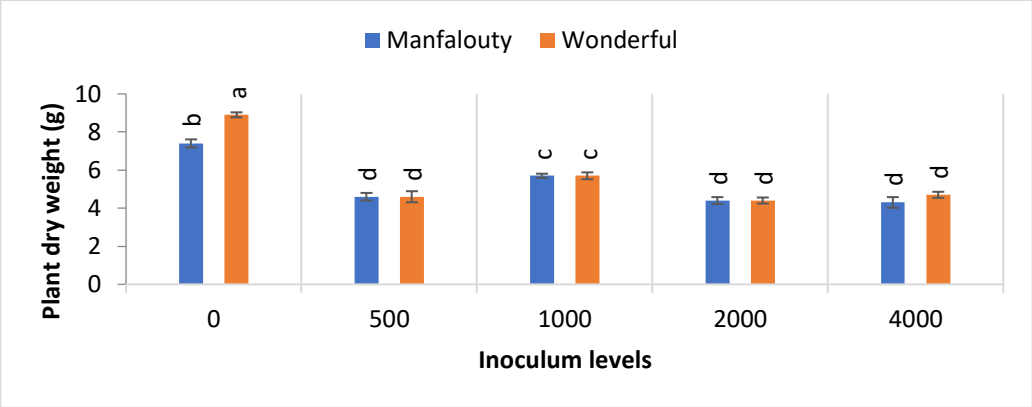

Figure 3. Plant dry weight of two pomegranate cultivars ('Manfalouty' and 'Wonderful') in response to different dosages of Meloidogyne incognita inoculum. Means ± SD followed by a common letter(s) within a column are not significantly different (P ≤ 0.05) according to ANOVA and Duncan's multiple range test.

Experiment 1: Effect of different dosages of M. incognita inoculum on chemical contents of pomegranate cvs 'Manfalouty' and 'Wonderful'

The results of the quantitative chemical analysis of total soluble carbohydrates, phenolics, tannins, nitrogen, phosphorus, and potassium in dry whole plant tissue in response to different dosages of nematode inoculum on two pomegranate cultivars are shown in Table 3. The percentage total soluble carbohydrate concentration decreased in response to increasing inoculum levels compared with the control plants, the highest percentage decrease being achieved at 4,000 J2/plant on 'Manfalouty'. In the 'Wonderful' cultivar, on the other hand, there was no significant reduction in total carbohydrate concentration in response to nematode infestation. Meanwhile, the total phenolic concentration in the cultivar 'Manfalouty' increased in response to nematode infestation more than in 'Wonderful', albeit not significantly. The greatest increase in phenolics occurred in 'Manfalouty' challenged with 500 J2/plant, compared with the control; a similar trend was observed in tannin concentration, although with no significant difference between different inoculum dosages in 'Wonderful' in particular. The concentration of N, P, and K decreased in response to increasing inoculum dosages of nematodes compared with the no-nematode control in both cultivars, with the decrease being more pronounced in 'Manfaloty than in 'Wonderful'. The overall reductions were more pronounced at 4,000 J2/plant in both cultivars, with the greatest reduction being recorded in K, especially at the highest level of inoculum.

Table 3. Effect of different levels of Meloidogyne incognita inoculum on chemical contents of pomegranate cultivars.

Inoc. level (J_2/pot)	Total carbo. (g %)	Total phenol (g %)	Tannins (g %)	N (g/100 g D.Wt.)	P (g/100 g D.Wt.)	K (g/100 g D.Wt.)
			Manfalouty			
0	10.3±0.17^{a}	6.2±0.17^{d}	3.7±0.13^{b}	2.84±0.05^{c}	1.32±0.01^{a}	4.45±0.02^{a}
500	9.9±0.15^{ab}	7.1±0.12^{a}	4.3±0.09^{a}	2.80±0.05^{cd}	1.28±0.01^{b}	4.34±0.04^{b}
1000	9.5±0.13^{b}	6.3±0.15^{cd}	3.7±0.09^{b}	2.77±0.03^{cd}	1.25±0.01^{b}	4.13±0.02^{c}
2000	8.7±0.13^{c}	6.4±0.13^{bcd}	3.8±0.12^{b}	2.69±0.01^{d}	1.18±0.01^{c}	3.73±0.02^{e}
4000	7.2±0.13^{e}	6.7±0.13^{abc}	4.0±0.16^{ab}	2.54±0.02^{e}	1.05±0.02^{e}	2.92±0.01^{h}
			Wonderful			
0	8.1±0.13^{d}	6.8±0.08^{ab}	4.1±0.20^{ab}	3.11±0.05^{a}	1.18±0.01^{c}	3.86±0.02^{d}
500	8.1±0.12^{d}	6.9±0.16^{a}	4.1±0.20^{ab}	3.09±0.02^{a}	1.17±0.01^{c}	3.74±0.01^{e}
1000	8.0±0.20^{d}	6.8±0.13^{ab}	4.1±0.20^{ab}	3.07±0.01^{a}	1.17±0.02^{c}	3.63±0.02^{f}
2000	8.0±0.13^{d}	6.8±0.15^{ab}	4.1±0.06^{ab}	3.03±0.02^{ab}	1.15±0.01^{c}	3.40±0.01^{g}
4000	7.8±0.12^{d}	6.9±0.16^{a}	4.1±0.16^{ab}	2.95±0.06^{b}	1.11±0.01^{d}	2.95±0.02^{h}
$F_{9,30}$	49.886	4.420	1.818	26.626	40.889	648.682
p	<0.001	<0.001	0.106	<0.001	<0.001	<0.001

Means±SD followed by a shared letter(s) within a column in each block are not significantly different (P ≤ 0.05) according to ANOVA and Duncan's multiple range test. Carbo=soluble carbohydrates

Experiment 2: Effect of inorganic and nano-fertilizers on reproduction of root-knot nematode on pomegranate cultivars

The fertilizers were applied twice as soil drenches at two-week intervals to plants inoculated with M. incognita (first application 1 week after inoculation) under greenhouse conditions (Table 4). Generally, the inorganic fertilizers and nano- fertilizers significantly suppressed the nematode multiplication parameters when compared with the no-fertilizer ("nematode only") controls, with the inorganic fertilizes being less effective at nematode suppression than the nano-fertilizers.

The nano-fertilizers significantly reduced the number of galls and embedded stages more than did the inorganic fertilizers on both cultivars. Moreover, there was no significant difference among the three tested nano-fertilizers (HF®, HFD®, and HFS®) in terms of the number of galls and embedded stages. The nano-fertilizer, HFD®, was highly effective at reducing the nematode final population compared with the control in both cultivars. However, the reduction in the nematode final population was not significant among the three nano-fertilizers on 'Wonderful'. Also, HF® and HFS® on 'Wonderful' resulted in excellent reductions in all nematode parameters paving the way for the lowest Pf/Pi value and greatest decrease in nematode numbers but the opposite appeared in response to HFS® on 'Manfalouty' compared with the control.

Among the three inorganic fertilizers tested, As™ showed the least effect on gall number and embedded stage number compared with the control. On the other hand, the most suppressive inorganic fertilizer in terms of reducing the number of eggs (compared with the control) was As™ in both cultivars.

Each of the fertilizers increased plant length (Fig. 4), fresh weight (Fig. 5), and dry weight (Fig. 6) of both cultivars grown in the presence of M. incognita, except for SphTM, which reduced plant dry weight (by -6.4%) on 'Wonderful' (Fig. 6). The growth-stimulatory effect, which, for plant length, exceeded the non-inoculated plants without fertilizer treatment, was especially noticeable in the case of the nano-fertilizer treatments, where HF® caused the highest percentage increases in each of the plant growth parameters of 'Wonderful', namely plant length (41.9%), plant fresh weight (129.1%), and plant dry weight (174.5%).

Table 4. The effect of three inorganic fertilizers, three nano-fertilizers, and one Meloidogyne incognita dosage on the nematode reproduction and multiplication on two pomegranate cultivars.

Treatment	Fertilizer	Nematode counts			Pf/Pi	Eggs/Egg mass
		Galls/Root	Embedded stages/Root	Final pop. (Emb. + in soil)		
				Manfalouty		
As™	Mineral	582±10.27^{c}	641±11.30^{c}	10201±161.88^{c}	2.6±0.03^{c}	321±5.65^{ef}
Sph™	Mineral	518±8.78d^{e}	544±9.35^{ef}	8971±136.59^{fg}	2.3±0.03^{ef}	372±4.69^{bcd}
Sp™	Mineral	503±11.95^{def}	578±13.74^{de}	9644±158.04^{de}	2.4±0.04^{de}	364±18.98^{bcd}
HF®	Nano	446±15.54^{g}	519±14.71^{fgh}	8769±129.85^{fg}	2.2±0.02^{fg}	360±7.40^{bcd}
HFD®	Nano	448±14.55^{g}	490±16.03^{gh}	8040±228.65^{h}	2.0±0.05^{h}	345±8.10^{def}
HFS®	Nano	452±15.19^{g}	487±4.17^{gh}	10057±116.33^{cd}	2.5±0.04^{cd}	349±3.54^{cde}
Nema only		1021±20.79^{b}	1277±25.98^{b}	12757±299.49^{b}	3.2±0.07^{b}	330±18.04^{ef}
				Wonderful		
As™	Mineral	542±9.54^{d}	588±10.56^{d}	9247±137.68^{ef}	2.3±0.05^{ef}	318±5.36^{f}
Sph™	Mineral	482±8.21^{efg}	522±9.24^{fg}	8798±131.82^{fg}	2.2±0.05^{fg}	385±4.69^{b}
Sp™	Mineral	468±11.25^{fg}	507±12.32^{fgh}	8582±177.48^{g}	2.2±0.05^{fg}	378±6.12^{bc}
HF®	Nano	440±14.37^{g}	479±15.59^{h}	7884±125.29^{h}	2.0±0.03^{h}	360±7.79^{bcd}
HFD®	Nano	447±16.29^{g}	476±17.94^{h}	7886±132.36^{h}	2.0±0.03^{h}	362±8.88^{bcd}
HFS®	Nano	445±11.86^{g}	482±12.96^{gh}	7977±93.03^{h}	2.0±0.03^{h}	365±6.52^{bcd}
Nema only		1355±5.94^{a}	1543±7.79^{a}	15219±147.96^{a}	3.8±0.06^{a}	413±7.41^{a}
$F_{13,42}$		426.021	558.432	161.173	148.533	7.618
P		<0.001	<0.001	<0.001	<0.001	<0.001

Means±SD followed by a shared letter(s) within a column in each block are not significantly different (P ≤ 0.05) according to ANOVA and Duncan's multiple range test. As™=Ammonium sulfate; Sph™=Superphosphate; Sp™= Potassium sulfate; HF®=Hyper Feed; HFD®=Hyper Feed Drip; HFS®=Hyper Feed Solo. Pf = final population; Pi = initial population

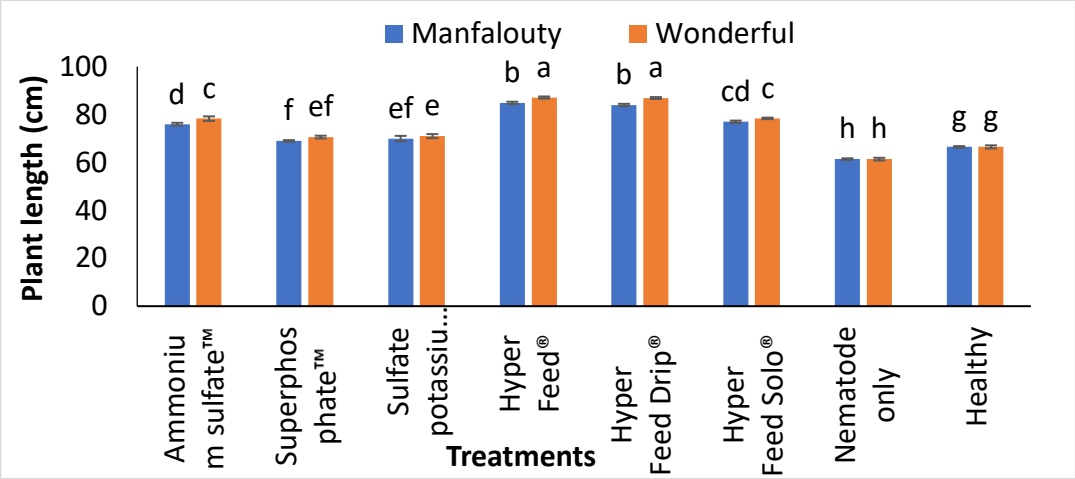

Figure 4. Length of two pomegranate cultivars ('Manfalouty' and 'Wonderful') infested with M. incognita and then treated with inorganic or nano-fertilizers. Means ± SD followed by a common letter(s) are not significantly different (P ≤ 0.05) according to ANOVA and Duncan's multiple range test.

The results of the quantitative chemical analysis of the two nematode-infested pomegranate cultivars following soil drench treatment with inorganic or nano-fertilizers are shown in Table 5. Total soluble carbohydrate concentration increased significantly in 'Manfalouty' plants treated with nano-fertilizers, especially with HF®, compared with the control and with similarly treated 'Wonderful' plants. The total phenolic concentration increased in 'Wonderful' when treated with the HFD® and HFS® nano-fertilizers, compared with the nematode-only and healthy (non-inoculated) control plants. A similar trend was apparent in tannin concentration (Table 5).

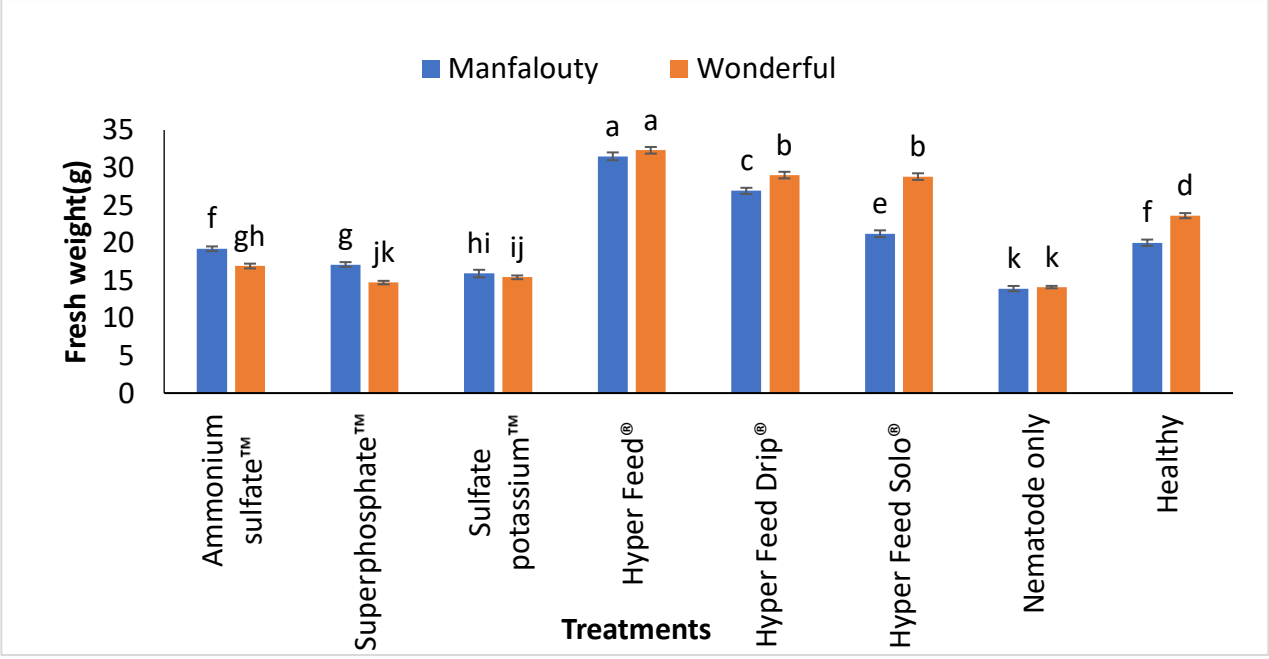

Figure 5. Plant fresh weight of two pomegranate cultivars ('Manfalouty' and 'Wonderful') infested with M. incognita and then treated with inorganic or nano fertilizers. Means ± SD followed by a common letter(s) are not significantly different (P ≤ 0.05) according to ANOVA and Duncan's multiple range test.

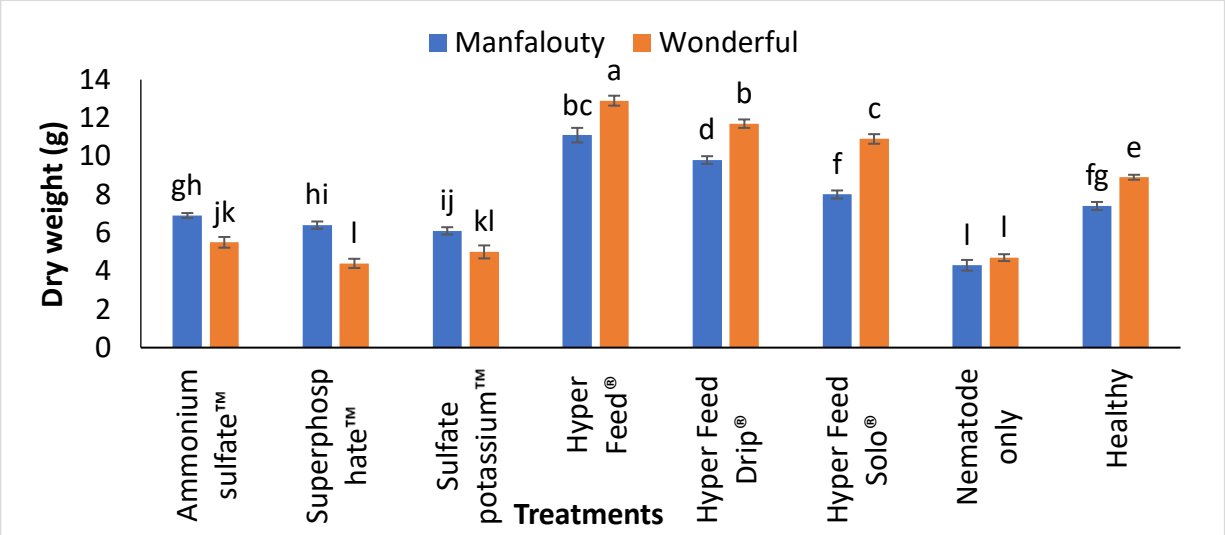

Figure 6. Plant dry weight of two pomegranate cultivars ('Manfalouty' and 'Wonderful') infested with M. incognita and then treated with inorganic or nano-fertilizers. Means ± SD followed by a common letter(s) are not significantly different (P ≤ 0.05) according to ANOVA and Duncan's multiple range test.

For each cultivar, nano-fertilizer treatment caused a significant increase in concentrations of organic chemicals (carbohydrates, phenolics, and tannins) relative to the inorganic fertilizers, but no significant difference in concentration of inorganic chemicals (N, P, and K). All nano-fertilizers exhibited a varying increase in N concentration. The highest values were achieved with HF® and HFD®. There was no significant difference between the effects on P concentration of the different treatments, but there was a significant difference between them and nematode-only and healthy control plants. For K concentration, there was no significant difference between inorganic and nano-fertilizers on either cultivar. A significant decrease in K was apparent when compared with the controls. The highest concentration of K occurred in the healthy plants of 'Manfalouty', whereas the lowest K concentration was exhibited in the nematode-only controls of both cultivars.

4. Discussion

Our results indicated that the two tested cultivars were good hosts for M. incognita at each of the nematode densities used. The observed increase in most nematode multiplication parameters in response to increasing nematode inoculum dosage supports the study's hypothesis that M. incognita negatively impacted pomegranate health. It agreed with previous reports on other hosts, suggesting a general weakening effect of this nematode species on the host plant. Varying reports have indicated the impact of different inoculum densities of different Meloidogyne species on various hosts (Ami and Shingaly 2020; Patel et al. 2020). Furthermore, the current findings revealed that raising the inoculum levels significantly reduced nematode multiplication (Pf/Pi) but the opposite was the case in terms of the number of galls and embedded stages. No significant relationship was observed between inoculum density and the number of eggs/egg mass. Nematode injury to plant growth parameters increased with increasing nematode inoculum dosage.

In terms of the chemical concentrations of the two cultivars, different dosages of M. incognita inoculum had distinct effects. The differential response in total soluble carbohydrate concentration between the two cultivars points towards potential genetic variations in anti-nematode response mechanisms. The greater decrease in carbohydrates could, in 'Manfalouty', suggest higher resource allocation toward defense or nematode-induced metabolic disruptions. Nematode infestation increased the concentrations of the metabolically related phenolics and tannins but decreased the content of total carbohydrates. The best-known component implicated in the susceptible/resistant response to nematodes is phenolic chemicals. The presence of phenolics in host tissues correlates with the degree of plant resistance (Schreiner et al. 2012; Castanheira et al. 2021; Tryfon et al. 2022). Plant tissues contain bound phenolics in the form of glycosides with minimal physiological and chemical activity. By secreting β-

glycosidases into host tissues, nematodes can break down glycosides into free phenolics (Masseneer 1964; Wilski and Giebel 1966).

Table 5. Effects of inorganic and nano-fertilizers on chemical contents of pomegranate cultivars Manfalouty and Wonderful infested with M. incognita.

Treatment	Fertili	Total carbo. (g %)	Total phenol (g %)	Tannins (g %)	N (g/100 g D.Wt.)	P (g/100 g D.Wt.)	K (g/100 g D.Wt.)
				Manfalouty			
As™	Mineral	8.5±0.17^{gh}	6.4±0.10^{fg}	2.6±0.16^{f}	3.80±0.09^{bc}	2.16±0.04^{a}	3.23±0.03^{de}
Sph™	Mineral	9.0±0.19^{ef}	6.7±0.11^{def}	3.2±0.07^{e}	3.74±0.03^{c}	2.20±0.05^{a}	3.24±0.03^{cde}
Sp™	Mineral	9.1±0.09^{ef}	6.8±0.09^{cde}	3.3±0.07^{e}	3.75±0.05^{c}	2.17±0.04^{a}	3.34±0.03^{cde}
HF®	Nano	10.3±0.17^{a}	7.0±0.13^{bcd}	4.2±0.05^{c}	3.87±0.05^{abc}	2.22±0.04^{a}	3.33±0.03^{cde}
HFD®	Nano	10.1±0.03^{ab}	7.3±0.12^{ab}	5.1±0.07^{b}	3.86±0.07^{abc}	2.23±0.02^{a}	3.39±0.06^{cd}
HFS®	Nano	10.2±0.08^{ab}	7.3±0.13^{ab}	5.3±0.05^{b}	3.81±0.06^{bc}	2.21±0.05^{a}	3.40±0.05^{c}
Nema only		7.2±0.13^{j}	6.7±0.13^{def}	4.0±0.16^{cd}	2.54±0.02^{f}	1.05±0.02^{c}	2.92±0.01^{f}
Healthy		9.5±0.19^{cd}	6.2±0.17^{g}	3.7±0.13^{d}	2.84±0.05^{e}	1.32±0.01^{b}	4.54±0.02^{a}
				Wonderful			
As™	Mineral	8.3±0.17^{h}	6.5±0.08^{efg}	3.1±0.07^{e}	3.92±0.03^{ab}	2.16±0.03^{a}	3.22±0.09^{d}
Sph™	Mineral	8.8±0.08^{fg}	6.7±0.08^{def}	3.7±0.11^{d}	3.81±0.05^{bc}	2.19±0.23^{a}	3.23±0.11^{de}
Sp™	Mineral	8.9±0.16^{efg}	6.8±0.11^{cde}	3.9±0.08^{cd}	3.82±0.03^{bc}	2.17±0.03^{a}	3.32±0.04^{cde}
HF®	Nano	9.3±0.13^{de}	7.2±0.07^{abc}	5.2±0.04^{b}	4.00±0.05^{a}	2.21±0.02^{a}	3.33±0.05^{cde}
HFD®	Nano	9.8±0.07^{bc}	7.5±0.19^{a}	6.2±0.08^{a}	3.99±0.06^{a}	2.22±0.08^{a}	3.39±0.05^{cd}
HFS®	Nano	9.9±0.27^{abc}	7.6±0.16^{a}	6.3±0.05^{a}	3.93±0.03^{ab}	2.20±0.06a	3.40±0.03^{c}
Nema only		7.8±0.12^{i}	6.9±0.16^{cd}	4.1±0.16^{c}	2.95±0.06^{e}	1.11±0.01^{c}	2.95±0.02^{f}
Healthy		8.1±0.13^{hi}	6.8±0.08^{cde}	4.1±0.20^{c}	3.11±0.05^{d}	1.18±0.01^{bc}	3.86±0.02^{b}
$F_{15,48}$		36.958	9.869	101.593	82.450	46.152	56.028
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means±SD followed by the same letter(s) within a column in each block are not significantly different (P ≤ 0.05) according to ANOVA and Duncan's multiple range test. As™=Ammonium sulfate; Sph™=Superphosphate; Sp™=Sulfate potassium; HF®=Hyper Feed; HFD®=Hyper Feed Drip; HFS®=Hyper Feed Solo. Carbo= soluble carbohydrates; Nema=nematode.

Tannins are used to create necrotic zones surrounding the embedded nematodes, particularly in resistant hosts. Tannin concentrations rose following challenge by nematodes, according to Farahat et al. (2012). Accordingly, the amount of tannins identified in infested plants may reflect the extent to which these host plants produce necrotic regions, walling-off the tissue surrounding nematode infection sites and negatively affecting multiplication of the pest.

The current findings revealed that M. incognita lowered the inorganic chemical concentrations (N, P, and K) of the pomegranate cultivars. When nematode inoculum density was increased, the highest rates of decrease in inorganic chemical concentrations were observed, compared with the control, especially on 'Manfalouty'. Few reports have dealt with inorganic chemistry analysis and its relationship to nematode infection. Shafiee and Jenkins (1963) discovered that root-knot-infested tomato roots had higher concentrations of N, P, and K than healthy tomato roots, whereas Oteifa and El-Gindi (1962) found that the foliage of M. incognita-infested tomato plants contained lower concentrations of N, P, K, Na, Ca, and Mg than healthy plants. Inorganic chemical concentrations in almond roots and leaves were significantly different between nematode (Meloidogyne javanica and M. incognita) -infested and non-infested plants (Nasr et al. 1980). They also reported that nematode infestation raised the concentrations of K, Ca, Mn, and Cu in roots and leaves, as well as P and N concentrations in leaves and roots. In the roots of eggplant, jasmine, and sour orange infested with M. incognita, Rotylenchulus reniformis, and Tylenchulus semipenetrans, respectively, Ca, Fe, Mg, P, K, N, and Zn concentrations were reduced relative to healthy controls. T. semipenetrans reduced inorganic chemical levels in their hosts more significantly than did M. incognita and R. reniformis. The elements most affected were Zn, Mg, Fe, and P (Mahfoud 2015).

Nanoparticle applications can suppress plant nematodes, either directly through their toxic effects toward nematodes (Elarabi et al. 2022) or indirectly by enhancing the production of plant defense compounds (El-Sherif et al. 2019). On both cultivars studied, the nano-fertilizers reduced the number of galls and embedded stages significantly more than did the inorganic fertilizers. Furthermore, there was no significant difference in the numbers of galls and embedded stages among the three nano-fertilizers

examined (HF®, HFD®, and HF S®). In addition, all nano-fertilizers outperformed the inorganic fertilizers in terms of plant growth parameters. These results corroborated the findings of El-Sherif et al. (2019), who discovered that soil-borne pests, such as the root-knot nematode M. incognita, were inhibited by nano-fertilizers, and that these fertilizers played a crucial role in enhancing plant yield. Also, Konappa et al. (2021) and Mohammadi et al. (2022) observed that nano-fertilizers improved the efficiency with which nutrients were used and resulted in reduced nutrient losses, reduced soil toxicity effects, and reduced negative consequences of over-application of fertilizers while also requiring fewer treatments.

In the current study, the nano-fertilizers significantly reduced the number of galls and embedded stages of the nematode more than the inorganic fertilizers on both cultivars. Moreover, there was no significant difference among the three tested nano-fertilizers (HF®, HFD®, and HFS®) in terms of the number of galls and embedded stages. Also, all three nano-fertilizers significantly increased plant growth parameters in infested plants more than the inorganic fertilizers. These findings supported those reported by El-Sherif et al. (2019), who noted that nano-fertilizers were highly effective in encouraging plant growth and had an inhibitory effect on hazardous soil microorganisms like M. incognita. Additionally, they claimed that applying inorganic or nano fertilizers on tomato plants cv. Giza 86 under the stress of M. incognita infestation decreased gall formation and egg masses and enhanced plant growth criteria. Our findings appeared to be consistent with those of Singhi et al. (2017), who discovered that nano-fertilizers provided the plant host with alternate metabolic reactions because of their larger surface area, which significantly improved photosynthesis, increased the production of dry matter and crop yield, and protected the plant host from various biotic and abiotic stresses.

5. Conclusions

This study investigated the potential of nano-fertilizers as a sustainable tool for managing the root-knot nematode, M. incognita, in pomegranate cultivation. Our findings demonstrated that nano-fertilizers significantly reduced gall formation and nematode reproduction compared with inorganic fertilizers on both cultivars tested. Additionally, they enhanced plant growth parameters, suggesting a dual benefit for pomegranate health and yield.

These results supported the potential of nano-fertilizers as a promising component of integrated pest management (IPM) programs for plant-parasitic nematodes in sustainable agriculture. Their targeted action, potential for enhanced nutrient uptake, and compatibility with other IPM practices, like resistant cultivars or biological control agents, warrant further investigation.

However, acknowledging the limitations of this study is crucial. Our experiments were conducted under controlled greenhouse conditions, potentially limiting generalizability to diverse field environments. Further research exploring nano-fertilizer efficacy across different pomegranate cultivars, soil types, and climatic conditions is needed. Additionally, the long-term effects and potential environmental implications of nano-fertilizer use require careful assessment.

Despite these limitations, this study paved the way for exciting possibilities in using nano-fertilizers to manage nematodes and promote sustainable pomegranate production. Future research focusing on field trials, specific mechanisms of action, and integrated management strategies can bridge the gap between controlled experimental settings and practical application in real-world agricultural systems.

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