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Induction of tomato plants resistance to *Meloidogyne incognita* infection by mineral and nano-fertilizer

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Nutrients are vital for plant growth as well as disease severity and control. Utilization of nanotechnologies in agriculture sectors is expected to be the ideal material to enhance plant resistance after perception of specific biotic or abiotic signals commonly referred to as induced resistance. Three mineral and nano-fertilizer particles were examined for their efficacy as a soil amendment in controlling the root knot nematode, *Meloidogyne incognita* on tomato in a greenhouse trial. A randomized complete block design layout was used for 9 treatments with 5 replications. Each plant was inoculated with 1,000 *M. incognita* eggs. Undoubtedly, the utilization of three fertilizers as mineral or nano-particles on tomato plants cv. Giza 86 under the stress of *M. incognita* infection significantly ($p \leq 0.05$) reduced the number of galls and egg masses as well as enhanced plant growth criteria. The application of nano-fertilizers was more effective than mineral ones. The nano fertilizer Zn oxide was the most effective treatment among all the treatments.

Key words: Disease severity, *Meloidogyne incognita*, nano-fertilizer, tomato, resistance.

INTRODUCTION

Meloidogyne incognita is the root-knot nematode (RKN), well-known as one of the most adverse phyto-pathogens all over the world (Trudgill and Blok, 2001), that cause severe damage on vegetable crops in tropical and subtropical areas (Sikora and Fernandez, 2005). Current research has publicized that plant resistance is alternative management too confirmed as safe to environmental system and economically viable means of controlling RKN. Plant resistance can be enhanced after perception of specific biotic or abiotic signals normally referred to induced resistance mechanism. The induced resistance can be extensive permanent and is often associated with a sensitization of basal defense responses (Pastor et al., 2013). Nutrients are important for plant growth and development as well as for phyto- pathogens disease severity and control (Agrios, 2005; Huber and Graham, 1999). Utilization of a small nutrients quantity can affect the vulnerability of plants to stress (Sultana et al., 2001). Zinc (Zn) and Iron (Fe) are recognized as significant micronutrients where it is considered as important components of several critical enzymes and participate in the biosynthesis of chlorophyll as well (Li et

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al., 2006). In recent years, nanotechnology tools have contributed to a great improvement in agriculture for sustainable development particularly in plant nutrients. Nano-fertilizer particles with small size and great surface area are characterized by competence of holding great quantity of nutrients, releasing it unhurriedly and steadily such that it facilitates uptake of nutrients matching the crop necessity without any linked toxicity of customized fertilizer inputs (Ghormade et al*.,* 2011). Nano-fertilizers are advantageous over conventional fertilizers as they raise soil fertility and yield quality parameters; they are nontoxic and less injurious to environment and humans thus they minimize cost and maximize profit (Naderi and Abedi (2012). Nano-particles with the size below 100 nm can be used as fertilizer for efficient nutrient management which is more eco-friendly therefore reducing environment pollution. These agricultural useable nano-particles developed by the helping of nanotechnology can be exploited in the value chain of entire agricultural production sectors (Joseph and Morrison, 2006). If nutrition benefits the host in the presence of nematodes, therefore, it is logical to suggest that healthy plant-based nutrient recommendations may not be adequate under nematode infested conditions and that nutrition can be used as a tool for nematode management (Melakeberhan, 1997). The current investigation aims to evaluate the efficacy of three mineral and nano-fertilizer particles as elicitors of systemic acquired resistance (SAR) on tomato plants against the stress of *M. incognita* infection under greenhouse conditions.

MATERIALS AND METHODS

Preparation of nematode inoculation

The root knot nematode, *M. incognita* identified culture was obtained from greenhouse of Nematology Research Unit, Agricultural Zoology Department Faculty of Agriculture, Mansoura University, Egypt, and harvested from infected root systems with very heavy root-knot nematode eggmasses of *Coleus blumei* (Kofoid& White) plants. These roots were washed by tap water, then soaked in 1.0% NaOCl and manually shaken for 60 s. After that, it was directly passed though sieves (500 mesh); the eggs were collected carefully after washing with tap water (Hussey and Barker, 1973). Finally, the numbers of egg were counted and used for inoculating tomato seedlings in the following experiment.

Source of mineral and nano-fertilizer particles

The tested fertilizers were obtained from Bio Nano Technology Company at Abd El Salam Aref Street –Mansoura, Egypt.

Nematicide

Oxamyl (Vydate 24%) Methyl-N-N-dimethyl-(N-(methyl) carbomycocyl)-1-Thioxamidate was used in the current investigation as a positive control for the following treatments.

Experimental design

The experiment was conducted under greenhouse condition (25±3°C) using the Randomized Complete Block Design with five replications for 9 treatments including: mineral-Zn (5g); nano-Zn oxide (5 mg); 13% mineral-Fe (5 g); nano-Fe oxide (5 mg); mineral Fe-Zn oxide(5 g); nano-Fe-Zn oxide (5 mg); nematode + oxamyl (0.3 mL/pot); nematode check (nematode alone); and plant without nematode (plant free-nematode) (Table 1). Forty five plastic pots (10 cm in diameter) filled in individually with 900 g autoclaved soils (clay: sand; 1:1, v/v) were transplanted with tomato seedlings cv. Giza 86 at age of 30 days of growth. One week later, 1000 eggs of *M. incognita* were added for 8 treatments with consist of 40 pots, also; one week later, all mineral and nano-fertilizer particles as well as the nematicide (oxamyl) were applied by mixing them with the soil.

Evaluation parameters for treatment efficacy

All plants related to each treatment were harvested and up-rooted; 45 days nematode inoculation, and both vegetative and root systems were used as fresh and dried tissues for the following efficacy evaluation analyses.

Plant growth parameters

The tomato plant growth parameters including; fresh shoot lengths; fresh shoot and root weights (FWt); and shoot dry weight (DWt) were measured and reorded (AOAC, 2005).

Determination of nematode disease severity

Tomato plant roots were examened for galls formation and egg masses numbers using stereomicrscope after staining in 0.01 acid fuchsin containing lactic acid (Byrd et al*.,* 1983).The disease severity (DS) for nematode infection including root galling (RGI) and egg masses (REI) were scored depending on a scale of 0 to 5 according to Taylor and Sasser (1978): 0= no galls or egg masses; 1= 1-2; 2= 3-10; 3= 11-30; 4= 31-100; and 5= more than 100 galls or egg masses per root system. Vermiform stages of *M. incognita* were extracted from soil by using the method of Goodey (1957) and then reproduction factor (RF) and rate of population increase (RPI) were calculated (Oostenbrink, 1966).

Biochemical analyses

Fresh vegetative leaf samples of each treatment were used for the determination of photosynthetic pigments (chlorophyll a, b, and a+b) based on the following equations (Goodwine 1965):

$$\text{Chlorophyll (a)} = \frac{12.7\ D\ 663 - 2.69\ D\ 645 \times V \times 10}{d \times 1000 \times 0.5}$$

$$\text{Chlorophyll (b)} = \frac{22.9\ D\ 645 - 4.68\ D\ 663 \times V \times 10}{d \times 1000 \times 0.5}$$

V = Acetone volume, D = Optical density read at wave length and d = / cm.

Table 1. Overview of tested nano-fertilizers particle size, surface area and effect.

Nano-Fertilizer	Particle Size	Surface Area	Effect	Ref.
Nano Zn oxide	20 nm	54 m^2-g^{-1}	ZnO NPs (40 nm-ml^{-1}) can reduce flowering period in onion by 12-14 days and even produce healthy seeds	Yang et al (2015)
Nano Fe oxide	<30nm	30 m^2- g^{-1}	The application of nano Fe-oxide (500 ppm) enhances *Vigna radiata* biomass	Dhoke et al. (2013).
Nano- Fe-Zn oxide	20 nm	30 m^2- g^{-1}	Application of nano Zn-Fe oxide (1.5 g/L^{-1}) increased about 17.40% from grain yield of *Triticum aestivum*	**Babaei** et al. (2017).

The dried leaves of tomato plants were ground and wet digested; they were subjected to the determination of nitrogen (N), phosphorus (P), potassium (K) contents, according to Kjeldahl methods (Anonymous, 1980) described by Pregl (1945), Jackson (1967) and John (1970).

The total phenol compounds were extracted and measured at 520 nm by spectrophotometer using chatichole as standard (Simons and Ross, 1971).

Statistical analysis

The obtained data were statistical analyzed to variance and then means compared according to Duncan (1955) and Gomez et al. (1984).

RESULTS

Table 2 shows the efficacy of different mineral and nano-fertilizers particles on tomato plants cv. Giza 86 against the stress of *M. incognita* infection (25±3ºC). It was clear that all fertilizers either as mineral or nano-particles significantly induced the reduction of the galls number and egg masses compared to untreated inoculated control (nematode check).

As a whole, the application of nano-fertilizers form is more effective compared to the mineral form. Nano-Zn oxide, Fe-Zn oxide and Fe oxide reduced galls by 94.1, 92.1 and 89.6% respectively. The nano-Zn oxide was the more effective fertilizer with significant differences than all other treatments for reduction of nematode root galls. On the other hand, the lowest reduction percentages were obtained by mineral Fe-Zn oxide fertilizer which reduced number of galls by 85.1%, as compared to untreated inoculated one. However, oxamyl as a nematicide showed the highest reduction percentage of number of galls by 96.0%.

All of the tested fertilizers in both mineral and nano-particles forms caused significant reduction in the number of egg-masses compared to untreated inoculated control (Table 2). The applications of nano-fertilizers were more effective than mineral fertilizers and the highest reduction activity was recorded by nano-Zn oxide (93.6%), followed by nano - Fe-Zn oxide (91.3%). Conversely, mineral fertilizer Fe-Zn was the lowest treatment, which reduced number of egg-masses by 83.8%. On the other hand, all of the tested fertilizers caused significant effect in reducing the number of second stage juveniles (J_2) in the soil compared with the untreated inoculated control (Table 2). The highest effect was obtained by application of nano-Zn oxide (33.6%) compared to oxamyl (36.6%) and nano-Fe oxide (42.0%), while mineral Fe-Zn showed the lowest effect (90.0).

Similarly, significant differences were noticed between egg masses indices (EI) of all tested fertilizers (3-4) and untreated nematode only(5). The application of nano-Zn oxide fertilizer had the lowest value of nematode reproduction factors (Rf) and rates of population increase (RPI) (0.08 and - 0.92); that of mineral fertilizer Fe-Zn showed the highest values of the same parameters (0.21 and 0.79), respectively, whereas oxamyl had the lowest values (0.07 and -0.93) in this respect (Table 2).

The data obtained in Table 3 illustrates the efficacy of different mineral and nano-fertilizer particles on growth of tomato plants cv. Giza 86 with infection of the root knot nematode, *M. incognita* under greenhouse condition (25±3ºC). Data indicate that all the tested fertilizers forms as mineral or nano-particles significantly increased in tomato growth parameters including shoot length, shoot fresh weight, root fresh weight and shoot dry weight compared to untreated inoculated control plants (nematode check). The application of nano-fertilizers was more effective than mineral ones; the utilization of nano-Fe oxide fertilizer showed the highest increase values of plant growth parameters that referred to 67.6, 85.5, 94.1 and 66.7% for shoot length, shoot FWt, root FWt and shoot DWt, respectively as compared to nematode check. Meanwhile, the other two nano-fertilizers, Zn-oxide and Fe-Zn oxide induced moderately increased values of shoot length (63.0 and 36.4%), shoot FWt (77.0 and 63.8%), root FWt (73.3 and 73.3%) and shoot DWt (62.1 and 43.7%), respectively.

The application of mineral fertilizer Fe-Zn achieved the lowest increased values of shoot length (30.3%), root FWt (66.3%) and shoot DWt (36.8%). Similarly, the systemic nematicide (oxamyl) had moderate but significant percentage values of plant growth parameters; 39.9, 59.9, 71.3 and 43.7% for shoot length, shoot FWt, root FWt and shoot DWt, respectively, compared to nematode alone. In the case of healthy tomato plants (plant without nematode), their growth parameters had

Table 2. Effect of mineral and nano-fertilizers particles on root galling, egg mass indices and reproduction of *M. incognita* infecting tomato plants cv. Giza 86 under greenhouse conditions (25±3ºC).

Treatments	Juveniles 2nd / 250 g of soil	Galls/ root system	RGI	Reduction (%)	Egg-mass / root system	EI	Reduction (%)	Final Population (PF)	Reduction (%)	RF	RPI
Mineral Zn (5 g)	82.0c	38.0[ed]	4.0b	91.3	35.3ed	4.0b	90.4	155.33ed	93.2	0.16	-0.84
Nano-Zn oxide (5mg)	33.6f	27.0[ef]	3.0c	94.1	23.3f	3.0c	93.6	83.9f	96.3	0.08	-0.92
Mineral Fe (5 g)	71.0d	53.0[bc]	4.0b	87.6	49.0c	4.0b	86.6	173.0c	92.4	0.17	-0.83
Nano Fe oxide (5 mg)	42.0e	45.0[cd]	4.0b	89.6	40.0d	4.0b	89.1	127.0d	94.4	0.13	-0.87
Mineral Fe -Zn (5 g)	90.0b	63.0[b]	4.0b	85.1	59.33b	4.0b	83.8	212.3b	90.7	0.21	-0.79
Nano Fe-Zn oxide (5 mg)	72.6d	35.0[cd]	4.0b	92.1	32.0e	4.0b	91.3	139.6e	93.9	0.14	-0.86
Oxamyl	36.6ef	19.0[f]	3.0c	96.0	15.0g	3.0c	95.9	70.6g	96.9	0.07	-0.93
Nematode check (Nematode alone)	701.0a	907.0a	5.0a	0.0	667.0a	5.0a	0.0	2275.0a	0.0	2.28	1.28

RGI=Root gall index; EI=Eggmass index. Reproduction Factor (RF) was calculated by dividing final nematode population (PF) by initial population (Pi), Pi=1000 eggs of *M. incognita*.
Rate of population increase (RPI) was calculated by (Pf-Pi) /Pi (Oostenbrink, 1966).
Values in each column followed by the same letter (s) are not significantl different according to LSD at 0.05% level.

Table 3. Effect of mineral and nano-fertilizers particles on tomato cv. Giza 86 growth parameters under the stress of *M. incognita* infection.

Treatments	Shoot length (cm)	Increase %	Shoot FWt (g)	Increase %	Shoot DWt (g)	Increase %
Mineral Zn- (5g)	58.3[bc]	55.1	50.6[b]	66.4	12.6[b]	44.8
Nano- Zn oxide(5mg)	61.3[ab]	63.0	53.8[ab]	77.0	14.1[a]	62.1
Mineral Fe (5g)	54.0[cd]	43.6	48.5[b]	59.5	12.9[b]	48.3
Nano Fe oxide(5mg)	63.0[ab]	67.6	56.4[a]	85.5	14.5[a]	66.7
Mineral Fe +Zn (5g)	49.0[d]	30.3	49.7[b]	63.5	11.9[b]	36.8
Nano Fe-Zn oxide(5mg)	51.3[d]	36.4	49.8[b]	63.8	12.5[b]	43.7
Oxamyl	52.6[d]	39.9	48.6[b]	59.9	12.5[b]	43.7
Nematode check (Nematode alone)	37.6[e]	0.0	30.4[c]	0.0	8.7[c]	0.0
Plant without nematode (Plant free-nematode)	65.0[a]	72.9	58.6[a]	92.8	14.8[a]	70.1

FWt= Fresh weight; DWt= Dry weight. Values in each column followed by the same letter (s) are not significantly different according to LSD at 0.05% level.

increased percentage such as 72.9, 92.8, 83.2 and 70.1%, respectively (Table 3).

The results of biochemical analyses show non-organic elements nitrogen (N), phosphorous (P) and potassium (K); photosynthetic pigments (chlorophyll a, b and a+b); and total phenols content in leaves of tomato cv. Giza 86 under greenhouse conditions (25± 3°C) after application of mineral and nano-fertilizer particles with infection of *M. incognita* (Table 4). Results revealed generally that all tested fertilizers had significantly positive values of N, P and K; and total phenol content, whereas a negative result recorded with total chlorophyll content was observed compared to nematode alone.

Among the tested fertilizers, the application of nano-Zn oxide surpassed other treatments with increased values of N (70.2%), P (59.6%); K (68.7%) and total phenol content (54.9%). On the other hand, the lowest values were recorded for mineral Fe-Zn with N (32.7%), P (39.0%), K (45.8%) and total phenol (31.7%) compared to nematode check (nematode alone). The positive control, oxamyl as a systemic nematicide recorded worthy considerable increased percentage values of N (34.1%), P (31.8%), K(35.9%) and total phenol content (35.7%), which showed significant increase from the nematode check. Relating to total chlorophyll contents in fresh tomato leaves under the induction of the tested fertilizers, the nano-particles form had reduced percentage ranging between 11.9 to 5.8% for nano-Fe-Zn oxide and nano-Zn oxide fertilizers, respectively (Table 4). Mineral fertilizers showed more reduction in chlorophyll contents than nano-particles. Meanwhile, the plant without nematode (free of nematode) showed reasonable values of

Table 4. Effect of mineral and nano-fertilizers particles on biochemical content of tomato leaves cv. Giza 86 under the stress of *M. incognita* infection.

Treatments	Biochemical analyses											
	Non-organic elements						Photosynthetic pigments					
	N%	Increase %	P%	Increase %	K%	Increase %	Chlo. a	Chlo. b	Chlo. a + b	% Reduction	T. phenol mg/100g	Increase %
Mineral Zn- (5g)	3.29^{abc}	60.5	0.526^{bc}	50.7	3.43^{abc}	60.3	0.493^{abcd}	0.309^{bcd}	0.802^{bc}	9.8	0.614^{a}	53.1
Nano- Zn oxide(5mg)	3.49^{a}	70.2	0.662^{a}	89.7	3.61^{a}	68.7	0.512^{ab}	0.325^{b}	0.837^{ab}	5.8	0.621^{a}	54.9
Mineral Fe (5g)	3.05^{c}	48.8	0.498^{cde}	42.7	3.22^{cd}	50.5	0.473^{bcde}	0.295cd^{ef}	0.768^{cde}	13.6	0.528^{c}	31.7
Nano Fe oxide(5mg)	3.34^{ab}	62.9	0.557^{b}	59.6	3.51^{cd}	64.0	0.501^{abc}	0.318^{bc}	0.819^{bc}	7.9	0.583^{ab}	45.4
Mineral Fe +Zn (5g)	2.72^{d}	32.7	0.485^{cde}	39.0	3.12^{de}	45.8	0.465^{cde}	0.287^{def}	0.752^{cde}	15.4	0.527^{c}	31.4
Nano Fe-Zn oxide(5mg)	3.13^{bc}	52.7	0.511^{cd}	46.4	3.30^{ab}	54.2	0.481^{bcd}	0.302^{bcde}	0.783^{bcd}	11.9	0.540^{bc}	34.7
Oxamyl	2.75^{d}	34.1	0.460^{e}	31.8	2.91^{e}	35.9	0.439^{e}	0.269^{f}	0.708^{e}	20.4	0.544^{bc}	35.7
Nematode check (Nematode alone)	2.05^{e}	-----	0.349^{f}	-----	2.14^{f}	-----	0.528^{a}	0.361^{a}	0.889^{a}	-----	0.401^{d}	-----
Plant without nematode (Plant free-nematode)	**2.53^{d}**	**23.4**	**0.472^{de}**	**34.9**	**3.03de**	**41.6**	**0.452de**	**0.280ef**	**0.732^{de}**	**17.7**	**0.500^{c}**	24.7

Means in each column followed by the same letter (s) did not differ at P≤ 0.05 according to Duncan multiple-range test

N (23.4%), P (34.9%) and K (41.6%) and total phenol content (24.7%) which in some cases had higher value of P (41.6%) and K (41.6%) than that of oxamyl (P, 31.9%; K, 35.9%).

DISCUSSION

The utilization of three fertilizers as mineral or nano-particles on tomato plants cv. Giza 86 under the infection of *M. incognita* significantly (p ≤ 0.05) reduced tested nematode parameters and improved plant growth criteria. The application of nano-fertilizers was more effective than mineral ones. The nano fertilizer Zn oxide was the more effective treatment with significant differences than all other treatments. These results confirm the results obtained by Singhi et al. (2017) who reported that nano-fertilizers are able to improve plant growth, yield and quality parameters by increasing nutrient efficiency, reduce wastage of fertilizers and economic cost of cultivation. Tarafdar et al. (2012) reported that nano-particles fertilizers increase nutrients use efficiency and minimize the economic costs of environmental protection as well as enhance plants growth by resistance diseases and improving plant stability by anti-bending and deeper rooting of crops. Our results are in accordance with the results obtained by Singhi et al. (2017) that, nano-fertilizers provide different metabolic reactions due to more surface area to the plant host which increases the rate of photosynthesis; it produces more dry matter and crop yield, and also protects plant host from different biotic and abiotic stress

Conclusion

The application of nano-fertilizers plays an important role in enhancing plant production as well as having inhibitory effects on soil injurious microorganisms such as the root knot nematode, *M. incognita* infection. The use of nano-Zn oxide for fertilization of tomato cultivations under greenhouse conditions can be recommended. It will enhance plant growth and protect *M. incognita* infection. At the same time it is better to use nano-Fe oxide additionally in parallel with nano-Zn oxide that can enhance plant growth parameters and keep plant healthy stronger. Moreover, it is effective to reduce the cost of fertilizer for crop production and also minimize pollution hazard. These results need to be further emphasized by conducting experiments under field conditions and conducting necessary soil and plant analysis before giving the final recommendation for inclusion in IPM programs.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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