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SELENIUM AND ITS EFFECT ON PLANT-PARASITE SYSTEM *MELOIDOGYNE ARENARIA – TINY TIM* TOMATOES

OLGA BAYCHEVA[1]; HARRY SAMALIEV[1*]; ZHANNA UDALOVA[2]; KOSTADIN TRAYANOV[1]; SVETLANA ZINOVIEVA[2]; GUNAR FOLMAN[3]
[1]*Agricultural University, BG-4000, Plovdiv, Bulgaria*
[2]*Severtsov's Institute of Ecology and Evolution RAS, Center for Parasitology, Moscow 119071, Russia*
[3]*Institute of Metallurgy and Metal Working RAS, Moscow 119991, Russia*

Abstract

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Effect of Se on the host-parasite system – tomatoes cv. *Tiny Tim – Meloidogyne arenaria* was studied. Seeds of tomatoes were moistened in water colloid solution of Se (nano solution) in concentration of 0.336 mg per L for 2 h. During the period of two months the development of the plants as well as the development of *M. arenaria* were observed. The following parameters of the plants and the parasite were measured: 1. Period for development of one *M. arenaria* generation; 2. Size of females; 3. Gall formation on the roots (after Barker,1985); 4. Plants height; 5. Fresh weight of the plants; 6. Size and fresh weight of the fruits. The positive effect after treatment with Se on the invasion of the plants was statistically proved. On the base of our experiments the changes of *M. arenaria* morphologic characteristics and the normal development of the plants can be explain through the effect of Se treatment on the plants–improvement of some plant innate mechanisms to *Meloidogyne* invasion.

Key words: Meloidogyne arenaria; invasion; Se (nano solution); tomatoes; development; plants

Introduction

Root knot nematodes belonging to *Meloidogyne* are among the most pathogenic plant parasitic nematodes of great economic importance. Their control is in the focus of the specialists and particularly application of methods with no nematicides.

Selenium (Se) is a trace element and interest in it has increased in the last decades. In trace amounts this element is essential and places important role in metabolism of humans and animals (Terry et al., 2000). However, at high dosages Se is toxic. That is why this element has been considered as toxic and environmental contaminant. It has been proved the role of Se in the prevention of atherosclerosis, some kind of cancer and altered immunological deficiency (Germ and Stibilj, 2007). Deficiency of Se was believed to cause some human diseases (Zahara et al., 2004). Selenium has been found in waters at Kesterson Reservoir California and has been shown as an agent responsible for mortality, developmental defects and reproductive failure in migratory aquatic birds and fish (Ohlendorf et al.,1986). The range between useful and toxic dosages is very narrow and specific for a given organism. Selenium has been shown in formation of important antioxidant enzymes, like glutathione peroxidase (Rotruck et al., 1973). It has been found the suppressive effect of Se on carcinoma and some symptoms of AIDS (Hori et al., 1997). The narrow margin between beneficial and harmful levels has been important implicate for human health. The toxicity of Se is due to its ability to replace sulfur (S) with Se in amino acids resulting in incorrect folding

**Corresponding author:* h.y.samaliev@abv.bg

of the proteins and consequently nonfunctional proteins and enzymes. Plants that accumulate Se may be useful as a Se-delivery system (in forage and crops) to supply the mammalian diet in many arias that are deficiency of Se. On the other hand the abilities of plants to absorb and sequester Se can also been harnessed to manage environmental Se contamination by phytoremediation (Terry et al.,1998). The special attribute of plants for phytoremediation of Se is their ability to convert inorganic Se to volatile forms predominantly dimethilselenide by process called phytovolatilisation (Terry et al., 2000).

Recently Se has been found to increase the antioxidant capacity of plants and to enhance their growth under UV stress (Pennanen et al., 2002). Selenium has increased yield under ambient radiation conditions (Germ et al., 2005). Plants respond to oxidative stress through increasing the enzymatic and non-enzymatic antioxidants (superoxide dismutase, catalase, ascorbic acid and carotenoids) (Kattab, 2004).

Some plants are able to accumulate Se, some other are non-accumulators. Selenium accumulators accumulate non-protein selenoamino acids, which contain most of the accumulated Se. They help and act in Se detoxication (Jackson et al.,1998 after Kattab, 2004). It has been proved that selenium accumulators are capable to exclude selenoamino acids from their proteins (Brown and Shrift, 1981). Selenium has been shown to be beneficial for the growth of many plants, both hyper accumulators and even non accumulators (Pilon-Smits et al., 2009). This effect may be due to Se influence on plant antioxidant capacity (Hartikainen, 2005).

Selenium has been found to exists in small amounts in microorganisms, plants, animals and humans. However the essentiality of Se to higher plants is still under debate (Terry et al., 2000). Xue et al. (2001) and Pennanen et al (2002) have proved the positive influence of Se on the growth of ageing seedlings. Senescence stress is partly affected on increasing of glutathione peroxidase activity. It also has been shown the ability of Se to regulate the water status of plants under drought conditions (Kuznetsov et al., 2003). Selenium concentration in plant accumulators are toxic to a wide variety of herbivores and pathogens (Quinn et al., 2008; Boyd et al., 2007). It has been proved the protective function of Se in *Brassica juncea* to fungi pathogens in the leaves (Hanson et al., 2003).

Uptake and accumulation of Se by plants depends on the chemical forms and concentration, soil factors (pH), salinity and $CaC0_3$ content, the identity and concentration of competing ions and the ability of plants to absorb and metabolize Se (Kabata Pendias, 1998).

Bacteria, fungi and algae have been shown to volatilize Se at rates higher than plants. Also Se content and volatilization depends on season of the year, water temperature and microbial biomass. According to some data the presence of bacteria in the rhizosphere of Indian mustard is necessary for volatilization of some Se forms (Terry et al., 2000).

On the base of above shown data is obviously the necessity to investigate the role of Se to higher plants in details. Future investigations will find new opportunities to use Se as a positive agent in increasing of innate plant resistance to unfavorable factors of the environment in different plant-parasitic systems.

The aim of our experiment was to study effect of the treatment with Se on the host- parasite system – *Meloidogyne arenaria – Tiny Tim tomato plants.*

Material and Methods

The experiments were carried out in three variants: 1. control – uninvaded and untreated (10 repetitions). 2. invaded and untreated (10 repetitions). 3. invaded and treated (10 repetitions). The working concentration of Se (nano-solution), kindly let us from G. E. Folman (Institute of Metallurgy and Metal-Working, RAS), was 0.336 mg per L. Seeds of tomatoes cv. *Tiny Tim* were moistened in water colloid solution of Se for 2 h and the seeds for the control were moistened in distilled water (DW). The treated seeds were left a few minutes under a laminar flow hood for drying, and then each seed was transferred in 18-cm diameter plastic pots previously filled with 1500 mL of steam-pasteurized loam soil mixed 2:1 (v/v) with washed sand. Pots were arranged on greenhouse benches in a randomized block design with ten replicates. The plants were watered as needed and fertilized weekly with 10 mL of commercial fertilizer (WUXALH® Super NPK fertilizer, 8-8-6 with micronutrients, 2.5 g / L). One month later a suspension of freshly hatched second-stage juveniles (J_{2S}) of *M. arenaria*, extracted from galled tomato roots with egg masses using the methods described by Whitehead and Hemming (1965), was adjusted to ~ 500 J_2s / mL and 4 mL of suspension per pot (2000 J_{2S}) were applied with an injection on 6 places in the rhizosphere. For the space of three months (April-June) the growth and the development of the plants as well as the development of *M. arenaria* (upon invasion) were observed. The greenhouse temperature during the cropping period was [16.5-31.6ºC (22.2ºC±3.4)] and humidity [26.5-79.1% (38.5%±91)]. The following parameters of the system were measured: 1. Time for development of one *M. arenaria* generation; 2. Size of females; 3. Gall formation on the roots (after Barker, 1985); 4. Plants height; 5. Fresh weight of the plants; 6. Size and fresh weight of the fruits.

The results obtained were analyzed by analysis of variants using SPSS 12.0 program.

Results

Development of *Meloidogyne arenaria*

According to the data obtained there was not found significant difference between the two variants *M. arenaria* without Se (*M. arenaria* – Se*)* and *M. arenaria* with Se (*M. arenaria* + Se*)*. The statistical processing of the results showed only a trend – elongation of the period for the development of one generation *M. arenaria* in respect to variant (*M. arenaria* + Se)*,* Table 1, Fig. 1.

Metric characteristics of *Meloidogyne arenaria*

The data about metric characteristics of *M. arenaria* (length of the female body –L- and diameter of the body – d -) are given in Table 2. Comparison between variant (*M. arenaria* – Se) and (*M. arenaria* + Se) showed the next: 1. The average values of L and d variant (*M. arenaria* – Se) are highest than those of variant (*M. arenaria* + Se). The difference of these characteristics (the smallest and the largest values of L) – variant *M. arenaria* – Se was 0.240 mm. This value for d (same variant) was 0.160 mm; 2. Data about differences between lowest and highest values of L was 0.345mm (variant *M. arenaria* + Se*)* and of d – 0.145 mm. According to our experiment the treatment of the plants with Se reflected on the measured parameters of *M. arenaria*.

Statistically processed metric characteristics of *M. arenaria* is given in Table 2a. The treatment with Se (variant *M. arenaria* + Se) influenced in the metric characteristics of *M. arenaria* particularly on the diameter. The average value of d (*M. arenaria* + Se) was lower compared with that of variant (*M. arenaria* – Se)*.* Statistically significant differences between variants (*M. arenaria* – Se) and (*M. arenaria* + Se) were found.

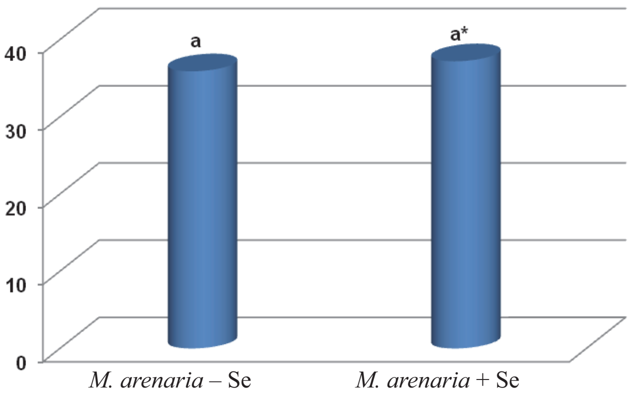

Figure 1. Effect of Period for development of one generation of *Meloidogyne arenaria* on tomato cv. *Tiny Tim* nontreated (-Se) and treated (+Se) with Selenium (nano-solution).

* Values followed by the same letter are not significantly different according by Duncan's Multiple Range Test ($P_{0.05}$)

Table 2
Metric characteristics of *Meloidogyne arenaria* (females)

Measured specimens (mm), n=20				
Variants	*M. arenaria* – Se*		*M. arenaria* + Se	
	Lenght	Diameter	Lenght	Diameter
1	0.630	0.450	0.700	0.400
2	0.700	0.430	0.620	0.400
3	0.680	0.470	0.670	0.430
4	0.720	0.400	0.736	0.480
5	0.610	0.500	0.590	0.390
6	0.750	0.520	0.645	0.390
7	0.800	0.470	0.850	0.500
8	0.780	0.510	0.590	0.400
9	0.730	0.510	0.600	0.410
10	0.830	0.500	0.870	0.490
11	0.850	0.550	0.900	0.500
12	0.740	0.530	0.567	0.510
13	0.800	0.520	0.825	0.390
14	0.825	0.530	0.710	0.410
15	0.690	0.470	0.800	0.410
16	0.700	0.470	0.650	0.535
17	0.720	0.500	0.555	0.500
18	0.750	0.510	0.620	0.430
19	0.800	0.560	0.760	0.400
20	0.810	0.560	0.750	0.420
Average	0.746	0.498	0.700	0.441

*Selenium (nano-solution) – nontreated (-Se) and treated (+Se)

Table 1
Effect of development of one generation of *Meloidogyne arenaria* on tomato cv. *Tiny Tim* nontreated (-Se) and treated (+Se) with Selenium (nano-solution)

Variants	Number of repetitions / Days after inoculation										
	1	2	3	4	5	6	7	8	9	10	Average
M. arenaria – Se	35	38	33	39	37	36	33	37	37	34	35.9
M. arenaria + Se	39	39	35	36	31	35	35	40	40	42	37.2

Table 2a
Metric characteristics of *Meloidogyne arenaria* (females)

Variants	L (μm)		d (μm)	
	$\bar{x}$	Sx	$\bar{x}$	Sx
M. arenaria – Se*	746 a**	1 9.1	498 a	18.7
M. arenaria + Se	700 b	18.5	441 b	17.7

*Selenium (nano-solution) – nontreated (-Se) and treated (+Se); ** Values followed by the same letter are not significantly different according by Duncan's Multiple Range Test ($P_{0.05}$)

Gall formation from *M. arenaria* on the roots of tomato

Gall formation on the roots is given in Table 3, 3a and Figure 2. Degree of galling on the roots of the experimental plants (variant *M. arenaria* – Se) was considerably higher expressed than degree of gall formation on the roots of treated plants (*M. arenaria* + Se). Fifty percents of invaded and untreated plants corresponded to 4th degree. Ten percents of galling in the same variant corresponded to the 5th degree of invasion (Table 3). The highest degree of invasion on the roots of the treated with Se plants (*M. arenaria* + Se) corresponded to 3th degree (30%), while 60% of the gall formation corresponded to 2th degree (Table 3).

By the statistical processing of the results efficiency of the treated plants with Se was made (Table 3a). The values up to 2 were considered as a very good efficiency. Our data showed considerably high value of efficiency (2.10) after the treatment (Table 3a).

Development of the plants

Height of the plants

The height of the experimental plants is given in Table 4 and Figure 3 (A and B).The average value of the plant height (control group) was considerably highest.The difference between height of the control group and that of the invaded and treated with Se was not significant, while comparison between invaded and untreated and invaded and treated plants showed statistically proved difference favorable to the treated with Se plants These results are well expressed for the space of the whole period of the experiment.

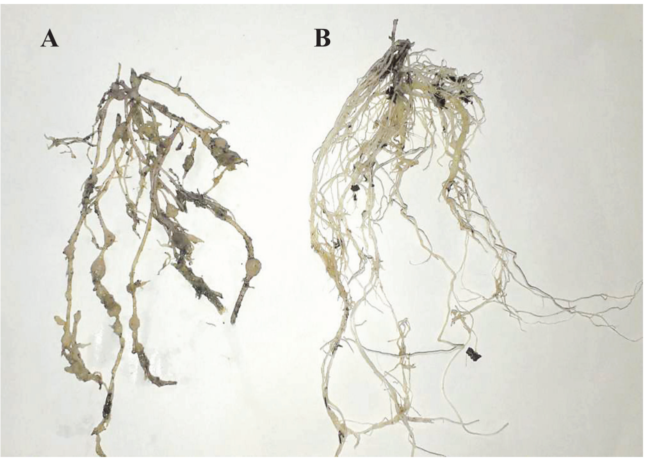

Fig. 2 Gall formation on the roots of tomato cv. *Tiny Tim*, A – invaded and untreated and B – invaded and treated with Se

Influence of Se on the height of the plants was observed for the space of the 60 days (Table 4). Differences among results for *M. arenaria* + Se and for uninvaded and untreated

Table 3
Degree of galling of *M. arenaria* on the roots of tomato cv. *Tiny Tim* treated (+Se) and nontreated (-Se) with Selenium (nano-solution)

Variants	Number of repetitions / Degree of galling										
	1	2	3	4	5	6	7	8	9	10	Average
M. arenaria – Se	3	4	4	4	5	3	2	4	4	3	50% 4 degree 30% 3 degree 10% 5degree 10% 2 degree
M. arenaria + Se	2	2	2	3	1	2	2	3	3	2	60% 2 degree 30% 3 degree 10% 1 degree

Table 3a
Effect of treatment of tomato cv. *Tiny Tim* with Se* against *Meloidogyne arenaria*

Variants	Degree of invasion / number of plants					Degree of invasion**
	I	II	III	IV	V	
M. arenaria – Se	0	1	3	5	1	3.80 a**
M. arenaria + Se	1	6	3	0	0	2.10 b

*Selenium (nano-solution) – nontreated (-Se) and treated (+Se);**Index up to 2 (after Barker, 1985) is considered to have very high efficiency; *** Values followed by the same letter are not significantly different according by Duncan's Multiple Range Test ($P_{0.05}$)

Table 4
Effect of Selenium (nano-solution) on height (cm) of the tomato cv. *Tiny Tim* plants 30 and 60 days after inoculation of *Meloidogyne arenaria*

Variants	Number of Repetitions										
	1	2	3	4	5	6	7	8	9	10	Average
					30th day						
M. arenaria – Se	25	30	28	20	35	40	32	29	43	45	32.7
M. arenaria + Se	32	38	35	43	40	30	45	40	45	43	39.1
No Invasion, No Se	45	33	35	47	38	50	40	45	50	42	42.5
					60th day						
M. arenaria – Se	30	25	33	29	35	42	35	38	45	45	35.7
M. arenaria + Se	43	45	30	35	46	55	48	55	52	45	45.7
No Invasion, No Se	50	45	40	50	52	44	55	49	46	53	48.6

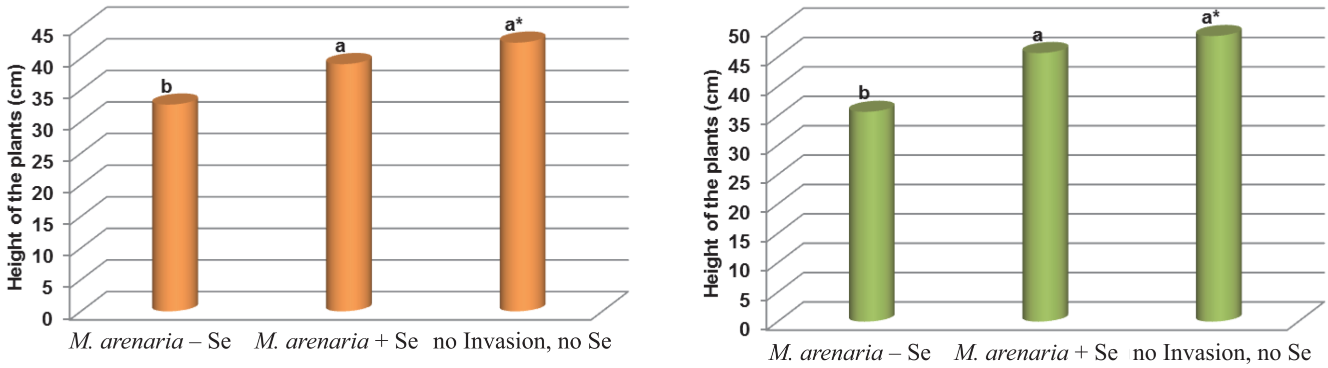

Fig. 3. Effect of Selenium (nano-solution) on height (cm) of the tomato cv. *Tiny Tim* plants in 30th (A) and 60th (B) day after inoculation of *Meloidogyne arenaria*.
* Values followed by the same letter are not significantly different according by Duncan's Multiple Range Test ($P_{0.05}$)

plants were not statistically significant both in 30th and 60th day (Figure 3A and B). The treatment with Se positively affected the height of the invaded and treated plants and their height almost leaded the height of uninvaded and untreated plants. These results for invaded and untreated (*M. arenaria* – Se) and invaded and treated (*M. arenaria* + Se) was statistically proved for the space of the vegetation period (Figure 3A,B).

Weight of the plants

The fresh weight of the plants was measured in the end of the experiment (on the 60th day) (Table 5A and Figure 4A). The differences among values showed a positive effect of Se

Table 5
Effect of Selenium (nano-solution) on fresh weight of the plants (A) and size of the fruits (B) of the tomato cv. *Tiny Tim* 60 days after inoculation of *Meloidogyne arenaria*

Variants	Number of Repetitions /										
	1	2	3	4	5	6	7	8	9	10	Average
					Fresh weight / g per plant (A)						
M. arenaria – Se	42	50	60	65	65	70	65	75	68	60	62.0
M. arenaria + Se	63	72	70	65	72	76	66	77	70	60	69.1
No Invasion, No Se	70	70	75	67	75	75	78	70	66	85	73.1
					Size / diameter in cm (B)						
M. arenaria – Se	2	2	2	2.3	2.5	2.5	2.5	2.7	2.7	2.8	2.4
M. arenaria + Se	2	2.3	2.5	2.5	2.6	2.6	2.7	2.7	3.0	3.0	2.59
No Invasion, No Se	2	2.4	2.5	2.5	2.7	2.7	2.7	3.0	3.0	3.0	2.65

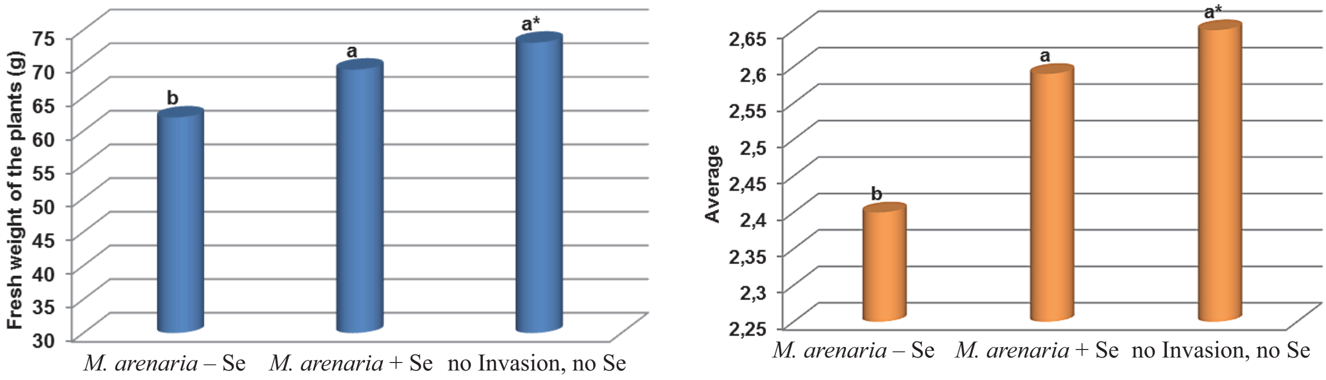

Figure 4. Effect of Selenium (nano-solution) on fresh weight of the plants (A) and size of the fruits (B) of the tomato cv. *Tiny Tim* 60 days after inoculation of *Meloidogyne arenaria*.

* Values followed by the same letter are not significantly different according by Duncan's Multiple Range Test ($P_{0.05}$)

on the plants after the treatment. The results obtained among invaded and untreated and invaded and treated plants are statistically significant (Table 5A and Figure 4A).

Treatment with Se of invaded with *M. arenaria* plants affected the fresh weight of the plants and it was almost equal to the fresh weight of the uninvaded and untreated plants (variant no Invasion no Se) . The statistical processing showed no significant difference between the two variants (*M. arenaria* + Se) and (no Invasion no Se) while the difference between the fresh weight of invaded and untreated and invaded and treated plants showed the positive effect of Se and this result was statistically proved (Figure 4A).

Size of the fruits

The size of the fruits is given in Table 5B and Figure 4B. The positive effect of selenium is particularly well expressed on the size of the fruits. The average values for diameter of the fruits between variants (*M. arenaria* – Se*)* and *(M. arenaria* + Se*)* were respectively favorable to the invaded and treated plants. Statistical processing proved significant difference between invaded and untreated and invaded and treated plants (Figure 4B).

Discussion

On the base of the results obtained the positive effect of Se on the invaded with *M. arenaria* tomato plants was shown. There were not found statistical differences of the investigated parameters of the plants between uninvaded and untreated and invaded and treated plants. Only unimportant differences in the framework of the statistical mistake were observed. However the positive effect after treatment with Se on the invaded with gall forming nematode plants was statistically proved. The positive effect of Se (0.336 mg per L) confirmed preliminary investigations about the positive results of low Se concentrations (5 µm to 10 µm) on the growth and development of the plants – both in plants-accumulators and non-accumulator (Pilon–Smiths et al., 2009; Hartikainen, 2005; Khattab, 2004). According to some experimental studies the positive effect of low Se concentrations have showed increasing of the respiratory potential of the plants from selenium treated seeds (Germ et al., 2005). Selenium has been found to increase the antioxidant capacity and stress-depending ability of lettuce plants and to enhance the growth of UV stressed plants (Pennanen et al., 2002). Increasing of the antioxidant capacity of the plants under unfavorable conditions (invasion with *M. arenaria*) explain the positive influence of the treatment with Se. The favorable influence of low Se dosage reflects on the reconstruction of the lipid and fatty acids composition of the plastids in the cell membrane (Renwei et al., 2013). Another important function of Se has been found to increase the photosynthetic rate of the plants (Renwei et al., 2013). These functions of Se increase the plant ability to develop enough well even under conditions of *Meloidogyne* invasion. Selenium concentration in plant accumulators has been proved to be toxic to a wide variety of herbivores and pathogens (Boyd, 2007). Also it has been found the protective role of Se in *Brassica juncea* to fungal pathogens in leaves (Hanson et al., 2003). There are not yet data about specific metabolic function of given selenium concentrations in plant pathogens including gall forming nematodes (*Meloidogyne spp.*). The close relation between glutathione peroxidase, selenium content and depending on them metabolic processes is common both for plants and animals. On the base of our results the changes of *M.arenaria* parameters could be explained through the effect of Se on the plants – improvement of some innate plant mechanisms for protection to *Meloidogyne* invasion.

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