*Acta Biologica Hungarica 54 (3–4), pp. 373–384 (2003)*

# EVALUATION OF CHANGES IN THE ELEMENT CONTENT AND BIOMASS OF INVADED WITH *MELOIDOGYNE ARENARIA* TINY TIM TOMATO PLANTS UNDER $NH_4VO_3$ TREATMENT

Anna Damianova,[1]* Olga Baicheva,[2] Delka Salkova,[2]
I. Sivriev[1] and Nadejda Lihareva[3]

[1] Institute of Nuclear Research and Nuclear Energy, Bulgarian Academy of Sciences, 72 Tzarigradsko Shosse Bld., Sofia 1784, Bulgaria; [2] Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 25, Sofia 1113, Bulgaria; [3] Central Laboratory of Mineralogy and Crystallography, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 107, Sofia 1113, Bulgaria

(Received: April 8, 2003; accepted: September 1, 2003)

The parasite-host system *Meloidogyne arenaria* – Tiny Tim tomato plants has been studied in order to investigate the influence of the process of invasion on the chemical composition and biomass of plants. The concentrations of seven chemical elements Cu, Zn, Mg, K, Na, Mn and Fe have been determined using AAS in controls and invaded plants, and their changes have been evaluated under treatment with $NH_4VO_3$ in three different concentrations – 0.01, 0.1 and 0.13 mg/100 ml $H_2O$. The process of treatment with $NH_4VO_3$ disbalances significantly the trace element content of plants. The lowest concentration (0.01 mg $NH_4VO_3$) causes bigger changes in the concentrations of Mn, Fe and Na in non-invaded plants. The highest concentration (0.13 mg $NH_4VO_3$) balances the content of the elements back to their levels in the control plants for the elements Zn, Fe and Na. The pure effect of the process of invasion with *Meloidogyne arenaria* on the biomass (leaves, stems, roots and total biomass) of Tiny Tim plants is expressed in a significant increasing, mainly due to the development of the parasites. After treatment with different concentrations of $NH_4VO_3$ the decreasing in the biomass of leaves, stems and roots is observed which reflects on the total biomass of plants. The concentration of $NH_4VO_3$ eliminates the unfavourable changes not only in the chemical content of plants but also in their biomass. It could be taken into consideration as an alternative method used instead of treatment with nematocides.

*Keywords:* Chemical elements – parasite-host system – Tiny Tim tomato plants – *Meloidogyne arenaria* – $NH_4VO_3$

## INTRODUCTION

It has been established that the quantity of elements in plants depends on their presence in the environment [2, 4, 10, 14]. The dynamic interaction between living organisms and chemical elements in the environment has become the focus of increasing study [2, 4]. Additional input from anthropogenic discharges shows two different ways of action – to disrupt the delicate balance attained within the ecosystem when the elements are toxic [1, 5, 19, 20] or to improve the productivity of agricultural plants when the elements are essential and used as fertilizers [15, 16].

* Corresponding author; e-mail: andam@inrne.bas.bg

The transfer of trace elements through the soil-plant system is of considerable interest in relation to crop productivity [15]. Some elements as Zn, Cu and Fe are essential and are involved in the biochemical processes of the organisms being incorporated into the plant tissues and becoming components or activators of many enzymes [2, 20]. At the same time they are a part of the anthropogenic activity which could disturb the harmony within the ecosystems [5, 15]. A significant problem is the interaction involving macro- and microelements N, P, K, Fe, Zn etc. in plants [8, 9, 12, 14] and its affection by high levels of vanadium which role is not yet clear.

Insufficient is also the existing information about the content of V and its role on parasite-plant system. Vanadium presents in the environment as a result of anthropogenic activity – mainly because of its wide industrial use and fuel burning [17]. Increasing content of vanadium in the humus soil is a base for its accumulation in plants [20]. Plants growing on soil contaminated with industrial dust may accumulate large amounts of vanadium [18]. Many of the agricultural plants accumulate vanadium in different quantities from 0.1 to 2.5 mg/kg dry weight [16]. Vanadium is required for plants growth [16] but its biotoxic effect is variable.

The root-knot nematodes (*Meloidogyne* spp.) are economically very important plant parasitic nematodes. At the end of 1988 the genus included about 60 species (61 species and two subspecies [7] some of them widespread in agricultural areas all over the world (*M. incognita, M. arenaria, M. hapla, M. javanica*)). At present the number of species belonging to *Meloidogyne* is about 80.

As obligate endoparasites they are pests for many cultivated and wild plants everywhere (in tropical, subtropical and temperate regions). They reduce the quantity as well as the quality of the yields [7, 13]. They cause formation of galls on the plant roots and consequently disruption of the vascular system.

The purpose of the present work is on one hand to investigate the influence of the process of parasite invasion with *Meloidogyne arenaria* on the element content of Tiny Tim tomato plants, and on the other hand to evaluate the effect of treatment with different concentrations of $NH_4VO_3$ in order to eliminate the unfavourable effects due to the invasion. Recently the pesticides for control of parasites becoming less effective and more expensive, and some different methods for controlling should be applied [11].

The investigation of the active role of vanadium in the plant-parasite system could result in a new approach for prevention of parasite invasion and used as an alternative way for influence on the process of invasion.

## MATERIAL AND METHODS

The experimental work has been carried out on Tiny Tim tomato plants invaded with *Meloidogyne arenaria* under laboratory conditions ($t = 18 – 20$ °C). The parasite *Meloidogyne arenaria* has been chosen for investigation because of its worldwide distribution and necessity of controlling their presence in the soil.

The Tiny Tim tomato plants were cultivated on soil sterilized by heating. The scheme of the experiment was as follows: $K_1$ – control (untreated, non-invaded plants); A, B, C – non-invaded, treated with 0.01; 0.1 and 0.13 mg $NH_4VO_3$/100 ml $H_2O$); $K_2$ – control (invaded, untreated); D, E, F – invaded, treated with the same concentrations of $NH_4VO_3$ as pointed above. All variants of the experiment were realized in three repetitions. The duration of the experiment was 2.5 months.

In order to evaluate the influence of parasite invasion during the vegetation, five measurements of the height and grow rate of the experimental plants were made [3] and the biomass of leaves, stems, roots and total biomass has been measured.

The treatment of the experimental plants has been made using $NH_4VO_3$ in three different increasing concentrations as pointed above added to the soil in 25 ml dose in four consecutive days in order to reach a final concentrations of 0.01, 0.1 and 0.13 mg $NH_4VO_3$.

By the end of the experiment plants were collected and the biomass of leaves, stems, roots and the total biomass were measured.

After homogenization, average samples of whole plants have been taken for analysis of chemical elements. The plant samples were heated at 525 °C until a complete ashing. Than they were treated with a mixture of nitric acid (Merck, Germany) and perchloric acid to the complete digestion. The solution was evaporated to dryness and the residue was diluted in 2% hydrochloric acid. Atomic absorption spectrometry (AAS) using Perkin Elmer 3030 (France) was used for determination of seven chemical elements Cu, Mn, Fe, Mg, Zn, K and Na. To receive comparative data, the samples were analyzed in duplicate. All results obtained are expressed in ppm dry weight.

## RESULTS

One of the main purposes of the present work was to investigate the influence of the process of invasion on the chemical content of plants. The mean concentrations (with standard deviations) of the elements (Cu, Zn, Mg, K, Na, Mn and Fe) in controls $K_1$ (non-invaded) and $K_2$ (invaded) are presented in Figs 1–3.

As it has been expected the process of invasion with *M. arenaria* influences the element content of plants. The concentrations of Cu, Mn, Zn (Fig. 1), Mg and K (Fig. 3) are lower in the invaded plants ($K_2$) in comparison with the control non-invaded plants ($K_1$). This is very well expressed in the content of Cu, where the concentration in invaded plants ($K_2$) is about three times lower (Fig. 1). An opposite tendency is observed in the content of Fe where its concentration under the process of invasion ($K_2$) increases 2.4 times (Fig. 2). No changes could be observed in the concentration of Na.

In order to evaluate the effect of treatment with $NH_4VO_3$ on the chemical content of plants under parasite invasion, three different concentrations – 0.01, 0.1 and 0.13 mg/100 ml $H_2O$ have been used. The results show two different reactions in the changes of chemical composition of the plants. In the D, E and F variants of invad-

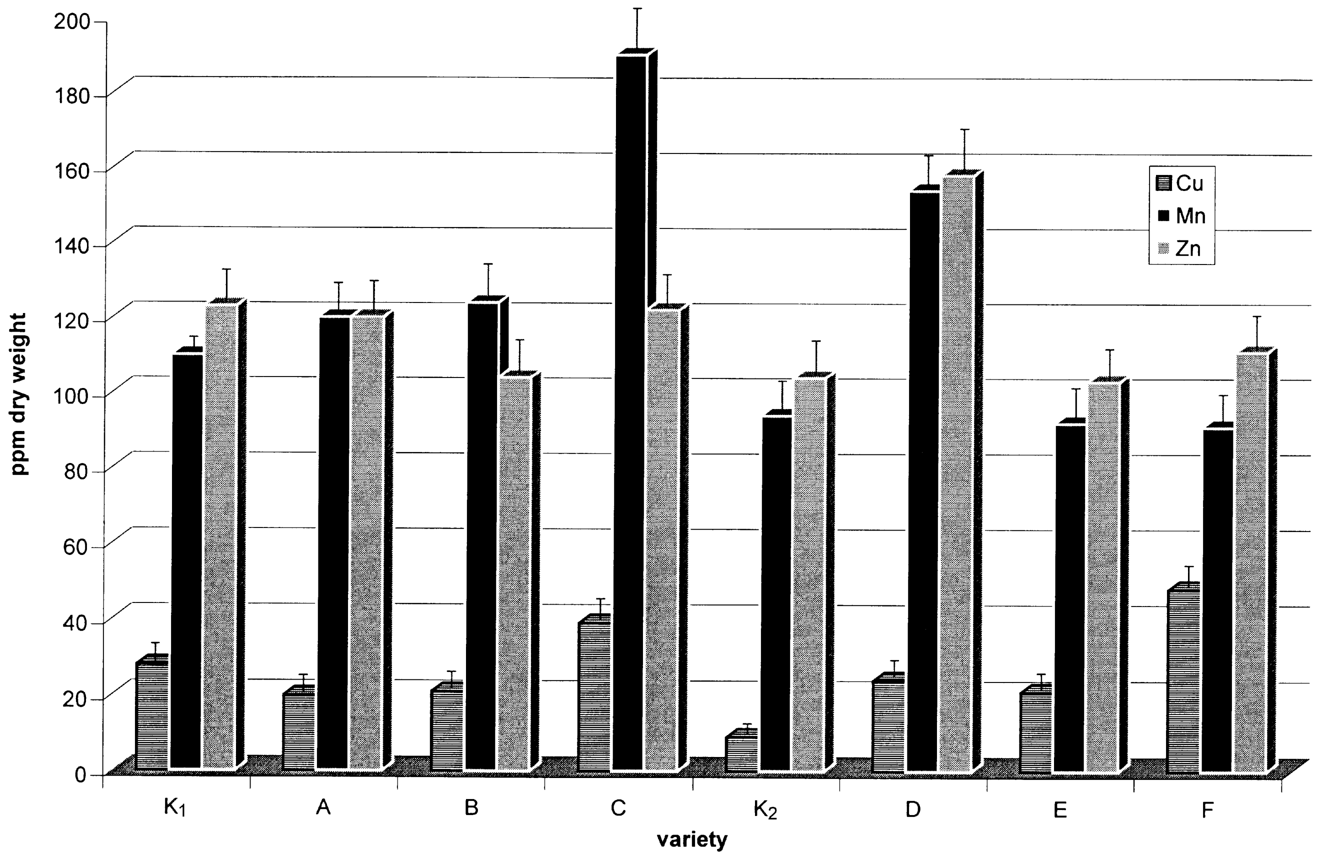


*Fig. 1.* Content of Cu, Mn and Zn (mg/kg dry weight ±SD) in Tiny Tim tomato plants; controls: $K_1$ – non-invaded, untreated; $K_2$ – invaded, untreated; A, B, C (non-invaded) and D, E, F (invaded) – treated with $NH_4VO_3$ in corresponding concentrations of 0.01; 0.1 and 0.13 mg/100 ml $H_2O$

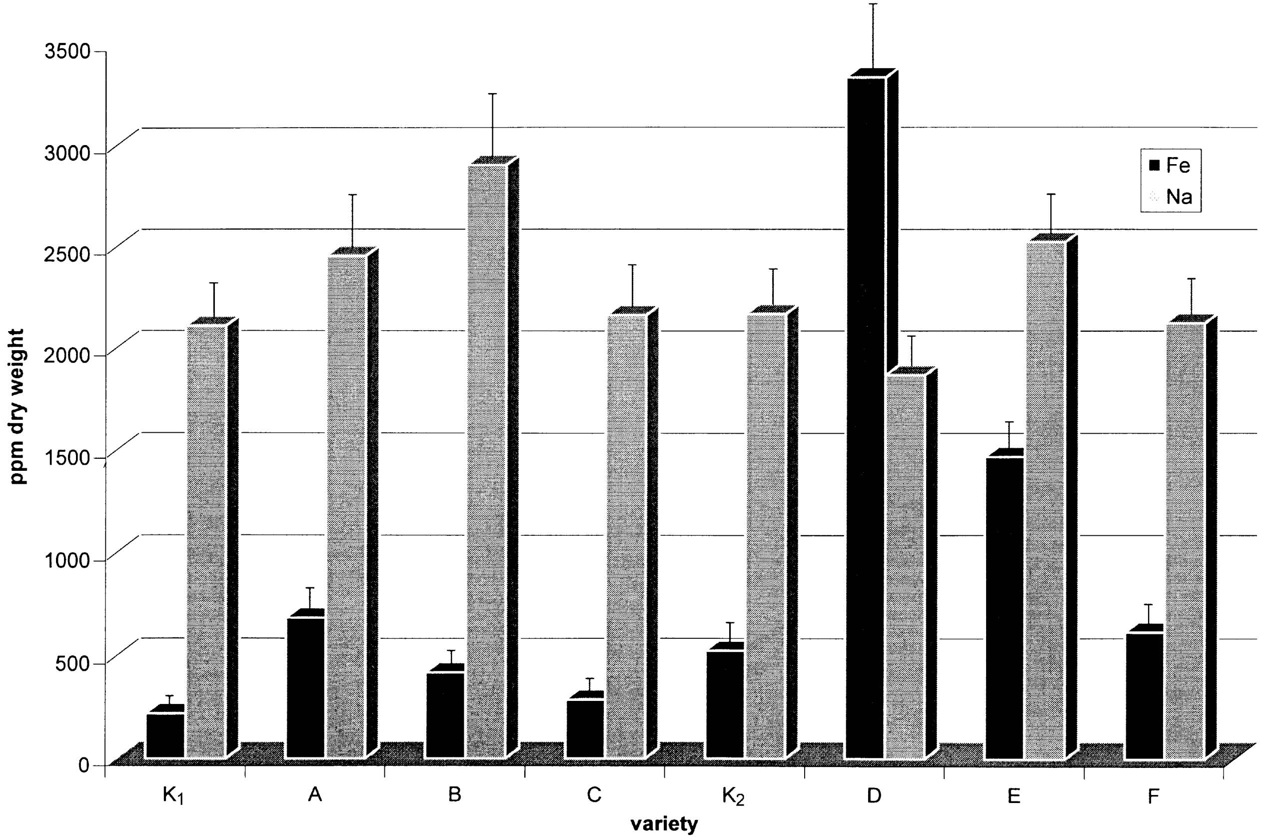


*Fig. 2.* Content of Fe and Na (mg/kg dry weight ±SD) in Tiny Tim tomato plants; controls: $K_1$ – non-invaded, untreated; $K_2$ – invaded, untreated; A, B, C (non-invaded) and D, E, F (invaded) – treated with $NH_4VO_3$ in corresponding concentrations of 0.01; 0.1 and 0.13 mg/100 ml $H_2O$

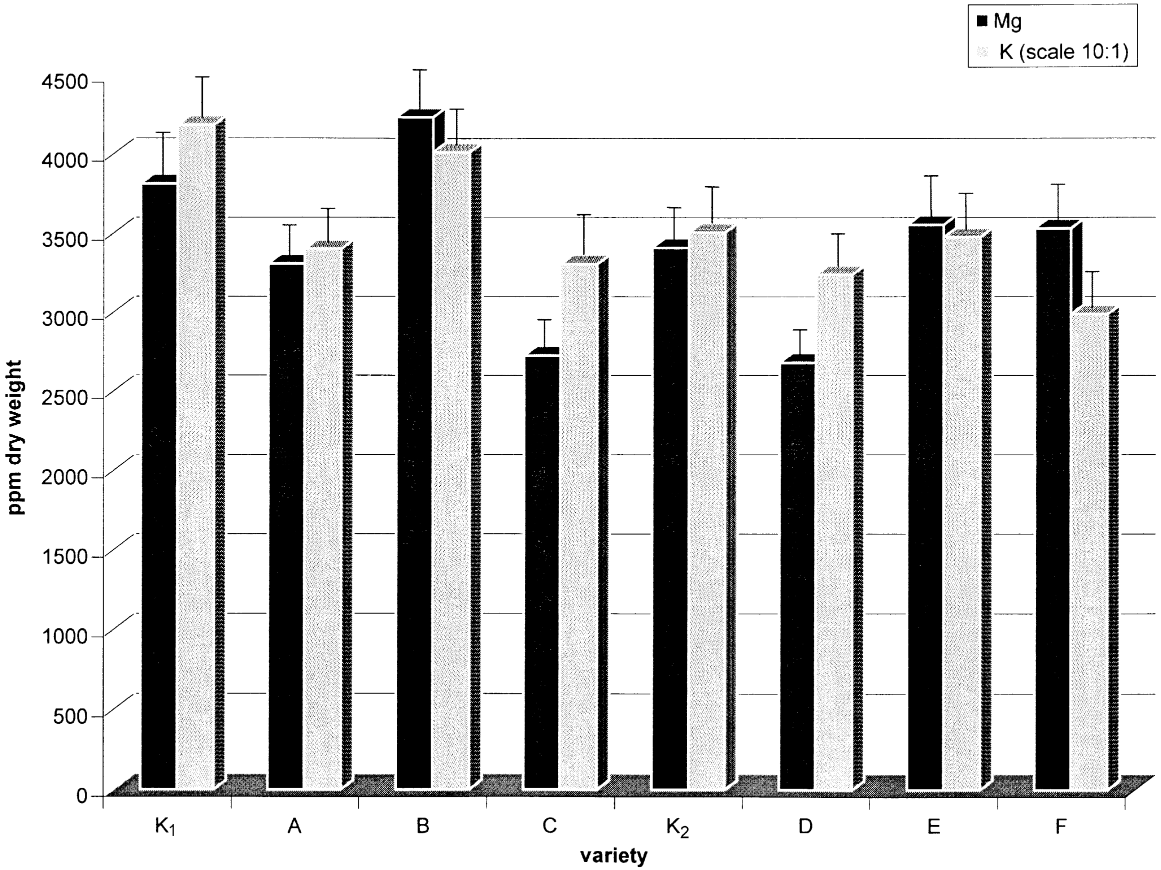


*Fig. 3.* Content of Mg and K (mg/kg dry weight ±SD) in Tiny Tim tomato plants; controls: $K_1$ – non-invaded, untreated; $K_2$ – invaded, untreated; A, B, C (non-invaded) and D, E, F (invaded) – treated with $NH_4VO_3$ in corresponding concentrations of 0.01; 0.1 and 0.13 mg/100 ml $H_2O$

ed plants, the concentrations of Mg, K (Fig. 3) and Na (Fig. 2) are lower in comparison with their concentrations in the non-invaded plants – A, B and C.

The concentrations of Cu (Fig. 1) in A, B, C and D, E, F variants under $NH_4VO_3$ treatment are very close but increase very significantly with the increasing of the vanadium concentrations. No changes in the concentration of Zn (Fig. 1) with the exception of its increasing in invaded plants treated with 0.01 $NH_4VO_3$ were observed.

The concentrations of Mn (Fig. 1) in non-invaded plants (A, B, C) extremely increase under the process of treatment with 0.13 mg $NH_4VO_3$. On the opposite, in the invaded plants its concentration decreases. This fact is in accordance with the results from other authors [19].

Biomass of plants was the second parameter investigated. By the end of the experiment the biomass of the plants has been determined and the results are given in Figs 4–7. The process of invasion influences the plant biomass. As a general result, the biomass leaves, stems and roots increases (Figs 4, 5, 6) in the process of invasion ($K_2$), in comparison with control non-invaded plants ($K_1$). It leads to an extremely increasing of the total biomass (Fig. 7), the fact due mainly to the increased mass of stems and roots (about 10 times). The relative part of the mass of leaves decreases up to two times.

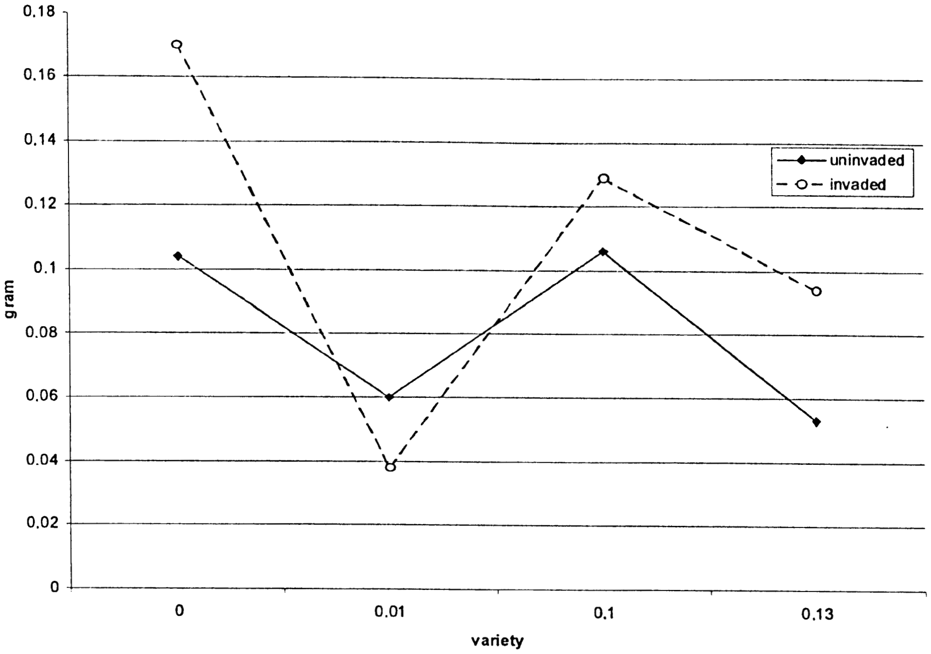


*Fig. 4.* Biomass of leaves of Tiny Tim tomato plants invaded with *Meloidogyne arenaria* and treated with $NH_4VO_3$/100 ml $H_2O$ in concentrations: 0 – corresponding to $K_1$ and $K_2$ varieties; 0.01 mg – corresponding to A and D varieties; 0.1 mg – corresponding to B and E varieties; 0.13 – corresponding to C and F varieties

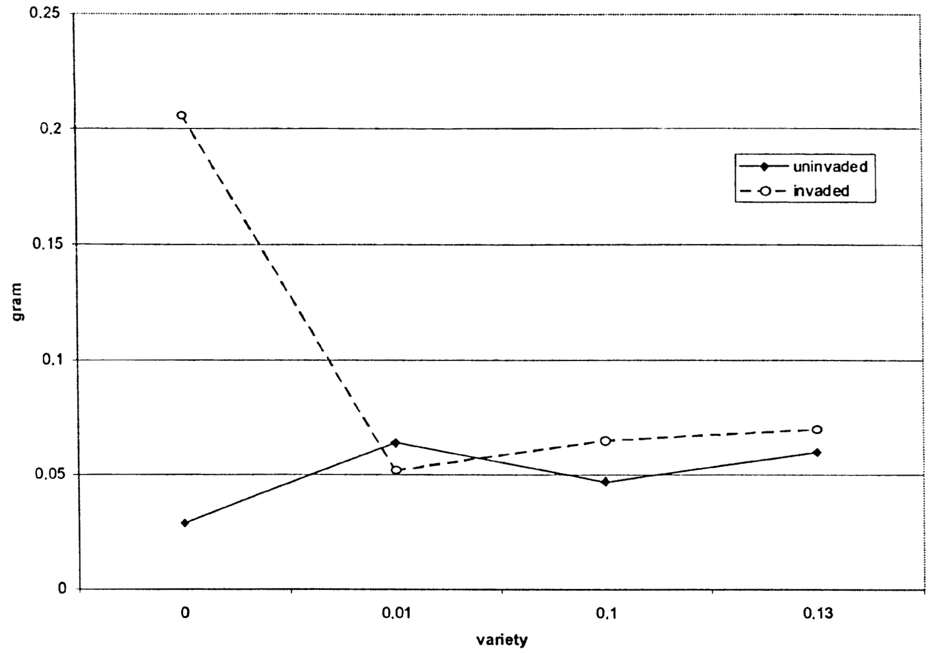


*Fig. 5.* Biomass of stems of Tiny Tim tomato plants invaded with *Meloidogyne arenaria* and treated with $NH_4VO_3$/100 ml $H_2O$ in concentrations: 0 – corresponding to $K_1$ and $K_2$ varieties; 0.01 mg – corresponding to A and D varieties; 0.1 mg – corresponding to B and E varieties; 0.13 – corresponding to C and F varieties

The treatment with $NH_4VO_3$ shows an opposite tendency in the biomass results. The biomass of stems and roots (Figs 5, 6) decreases, but that of leaves increases (Fig. 4) and it is close to this in control plants ($K_1$). This is very well expressed when a concentration of 0.13 mg vanadate is used for treatment (Fig. 7).

## DISCUSSION

The great number of root-knot nematodes (*Meloidogyne* spp.), their worldwide distribution and economic importance require their detail investigation in different aspects including the chemical content of plants. Moreover the infected plants are very susceptible to infections by other organisms – bacteria and fungi.

The process of invasion of Tiny Tim tomato plants with *Meloidogyne arenaria* influences the chemical composition of plants. Seven chemical elements (Cu, Zn, Mn, Fe, Mg, K, Na) considered to be of biological and high environmental importance have been determined.

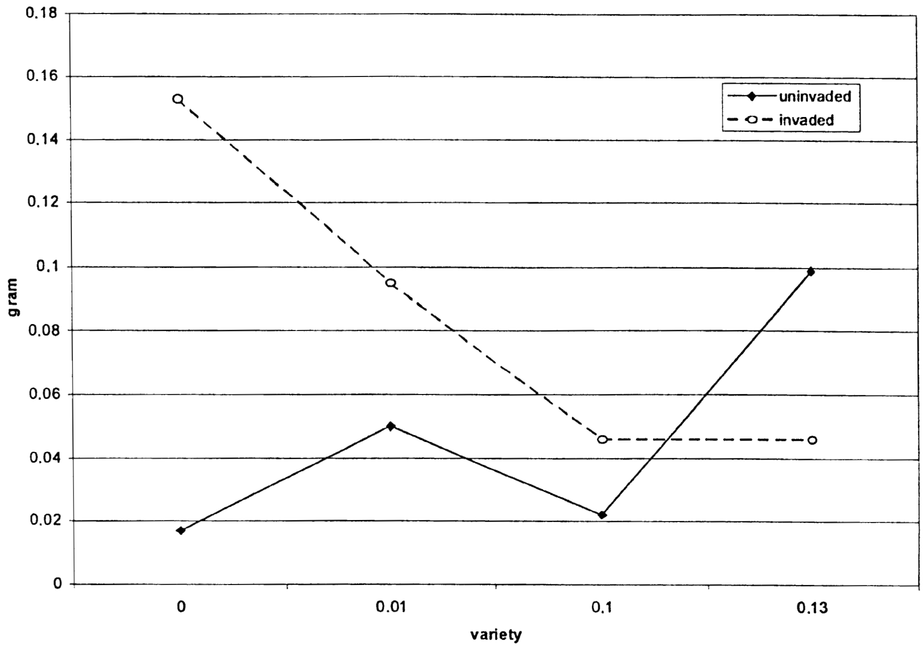


*Fig. 6.* Biomass of roots of Tiny Tim tomato plants invaded with *Meloidogyne arenaria* and treated with $NH_4VO_3$/100 ml $H_2O$ in concentrations: 0 – corresponding to $K_1$ and $K_2$ varieties; 0.01 mg – corresponding to A and D varieties; 0.1 mg – corresponding to B and E varieties; 0.13 – corresponding to C and F varieties

The greatest change (decreasing) is observed in the concentration of Cu (Fig. 1) and increasing of the concentration of Fe (Fig. 2). All other elements show slightly decreasing in their concentrations under the process of invasion.

Interesting result is that the treatment with the lowest concentration of vanadium causes significant increasing of Fe concentration in non-invaded plants. This fact is observed by other authors [2] and could find its explanation in some compensatory mechanisms. The treatment of experimental plants with $NH_4VO_3$ in three different concentrations shows different reactions as far as the element content is concerned. The treatment with lower concentration of vanadate (0.01 mg) shows a bigger changes on the chemical content of invaded plants in comparison with the treatment with the highest concentration (0.13 mg) mainly in the content of Fe, Zn, Mn and Na.

The treatment with 0.13 mg $NH_4VO_3$ acts in the opposite way – the concentrations for more of the elements (Mg, Zn, Mn, Fe, Na) are back to the concentrations of the control plants ($K_2$) with the exception of Cu and K.

Mainly the treatment with 0.13 mg vanadate reduces the development of *Meloidogyne arenaria* and could be explored as an alternative way instead of nematocide treatment. In this aspect we analyzed in details what is the effect of treatment with

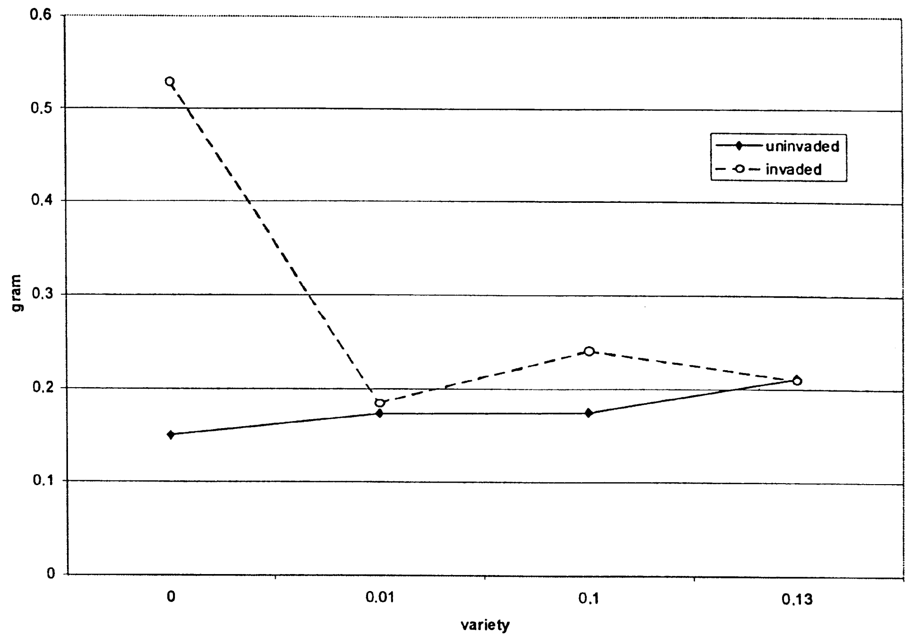


*Fig. 7.* Total biomass of Tiny Tim tomato plants invaded with *Meloidogyne arenaria* and treated with $NH_4VO_3$/100 ml $H_2O$ in concentrations: 0 – corresponding to $K_1$ and $K_2$ varieties; 0.01 mg – corresponding to A and D varieties; 0.1 mg – corresponding to B and E varieties; 0.13 – corresponding to C and F varieties

$NH_4VO_3$ on the balance of elements in invaded plants against their balance in control untreated plants ($K_1$).

For this purpose we obtained the relative alteration of the concentrations of elements in D, E and F against $K_1$:

$$\frac{D}{K_1}, \frac{E}{K_1} \text{ and } \frac{F}{K_1}.$$

Using the method of the smallest squares we try to find the case when the sum of the square declination of these alterations against $K_1$ has the smallest value. In this case the concentrations of elements will be closer to these in controls $K_1$. This is shown in Table 1. From Table 1 it is obvious that the sum of the square declination shows the smallest value in the process of treatment with 0.13 mg concentration of vanadate, i.e. this concentration restore the content of the elements closer to the controls.

The damages from the root-knot nematode *Meloidogyne arenaria* are limiting factors in the production of crops [11, 13]. According to the results obtained, the process of invasion enhances rapidly the total plant biomass mainly due to the part of stems and roots, where the parasites locate the biggest structural changes (Fig. 7). The following relative reduction of the biomass of leaves (Fig. 4) causes unfavorable changes in the plant development probably because of the influence on the process of respiration, nutrition and photosynthesis. The treatment with the concentration of 0.13 mg vanadate eliminates these changes and recovers the normal ratio in the biomass between the different parts of the plants.

*Table 1*

Declinations of relative alterations in the concentration of elements between the control plants ($K_1$) and the experimental plants (D, E, F)

| Δ | Cu | Mn | Zn | Fe | Na | Mg | K | $\Sigma \Delta^2$ |
|---|---|---|---|---|---|---|---|---|
| $\frac{D - K_1}{K_1}$ | –0.14 | 0.40 | 0.28 | 14.0 | –0.12 | –0.30 | –0.23 | 194.4 |
| $\frac{E - K_1}{K_1}$ | –0.27 | –0.16 | –0.17 | 5.66 | 0.19 | –0.07 | –0.17 | 32.2 |
| $\frac{F - K_1}{K_1}$ | 0.68 | –0.18 | –0.10 | 1.78 | 0.00 | –0.07 | –0.29 | 3.76 |

From the results obtained a general conclusion could be drawn for positive effect of the $NH_4VO_3$ in concentration 0.13 mg/100 ml $H_2O$ which eliminates the unfavorable changes caused by the invasion with *Meloidogyne arenaria* on Tiny Tim tomato plants, recovers the normal chemical content and the biomass of plants, and could be taken into consideration as an alternative way instead of the classical method of nematicide treatment which is decreasing recently due to the environmental concerns [6, 8]. Mainly the economic importance of the parasite balance requires application of effective methods for nematode control. It is very important to develop novel methods using biological specificity of the plant parasitic nematodes to some elements.

## REFERENCES

1. Antonovich, I. (1970) Heavy metals tolerance in plants. *Adv. Ecol. Res. 7,* 1–85.
2. Arnon, D. (1962) Role of microelements in plants nutrition. In: *Microelements.* Publ. Foreign Lit., Mosow, p. 158.
3. Baicheva, O., Salkova, D., Damianova, A. (2002) Influence of $NH_4VO_3$ on the invaded with *Meloidogyne arenaria* plants: Growth and development of the host. In: *Proc. Mat. 10$^{th}$ Congress of Bulg. Microbiol. Soc.*, 255 (in press).
4. Bowen, H. J. (1979) *Environmental Chemistry of the Elements.* Academic Press, London–New York, p. 315.
5. Cothenie, A., Dhaese, A., Camerlynck, R. (1976) Plant quality response to the uptake of polluting elements. *Qual. Plantarium 26,* 293–319.

6. Dusenbery, D. B. (1974) Analysis of chemotaxis in the nematode *Caenorhabditis elegans* by countercurrent separation. *J. Exp. Zool. 188,* 41–47.
7. Eisenback, J. D., Hirschmann, H. (1991) Root-knot nematodes: *Meloidogyne* species and races. In: Nickle, W. R. (ed.) *Manual of Agricultural Nematology*. Marcel Dekker, New York, pp. 191–274.
8. Hudson, T. G., Faulkner, G. (1964) *Vanadium: Toxicology and Biological Significance.* Elsevier, Amsterdam.
9. Myron, D., Givand, S. Nielsen, F. (1977) Vanadium content of selected food as determined by flameless atomic absorption spectroscopy. *J. Agric. Food. Chem. 25,* 297–300.
10. Perry, R. N. (1994) Studies on nematode sensory reception as a basis for novel control strategies. *Fundamental and Applied Nematology 17,* 199–203.
11. Rodriquez-Kabana, R., Robertson, D. Weaver, C. Wells, I. (1991) Rotations of bahiagrass and castorbean with peanut for the management of *M. arenaria. J. Nematology 23* (Suppl.), 658–661.
12. Soremark, R. (1967) Vanadium in some biological specimens. *J. Nutrition 92,* 183–195.
13. Taylor, C. R., Rodriquez-Kabana, R. (1999) Population dynamics and crop yield effects of nematodes and white mold in peanuts cotton and velvet beans. *Agric. Syst. 59,* 177–191.
14. Treshow M. (1988) (ed.) *Air Pollution and Plant Life*. John Wiley & Sons. New York
15. Tyler, G. (1972) Heavy metals pollute nature may reduce productivity. *Ambio 1,* 52–59.
16. Underwood, E. I. (ed.) (1977) *Trace Elements in Human and Animal Nutrition*. Academic Press, New York.
17. Wangen, L. E., Turner, F. B. (1980) Trace elements in vegetation downwind of a coal-fired power plant. *Water, Air, Soil Pollut. 13,* 99–108.
18. Welsh, R. M., Carry, E. (1975) Concentration of chromium nickel and vanadium in plant materials. *J. Agric. Food. Chem. 23,* 479–482.
19. Wlasuk, P. A. (ed.) (1980) *Microelements in the Environment.* Naukova dumka, Sofia, p. 268.
20. Zoller, W. H., Gordan, G. E., Gladney, E. S., Jones, A. G. (1973) In: Kothny, E. L. (ed.) *Trace Elements in the Environment*. Washington, DC.