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Foliar Application of Selenium under Nano Silicon on *Artemisia annua*: Effects on Yield, Antioxidant Status, Essential Oil, Artemisinin Content and Mineral Composition

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Abstract: The unique biological properties of *A. annua* have stimulated the research on its cultivation in different regions of the world. In this study, the effect of the Se and nano-Si supply on the yield, biochemical characteristics and mineral content of *A. annua* was investigated. Growth stimulation and a significant increase in the antioxidant status were recorded under Se and nano-Si foliar application. A decrease in the number of essential oil components and significant changes in the essential oil amount and composition led to significant phenophase shifts: nano-Si significantly stimulated eucalyptol and artemisia ketone accumulation and decreased germacrene D production, whereas Se demonstrated the opposite effect. A joint Se and nano-Si supply significantly decreased the camphor content and increased artemisia ketone and artemisinin levels by 1.3–1.5 times. Se/Si supplementation affected the macro- and microelements content, causing either a redistribution of leaves/stems elements (Al, Li and Zn) or a significant decrease in Ca, Mg, K, B, Cu, Fe and Mn concentrations in leaves, with no signs in growth inhibition or a decrease in the photosynthetic pigments content. The biofortification of *A. annua* with Se singly or in combination with nano-Si resulted in the synthesis of products with a Se content of as much as approximately 16% of the daily adequate Se consumption level (ACL) when using 5 g day^{-1} as a spice, or 36% of ACL when using 50 mL of tea infusion (1:2, *v*/*w*). The results indicated a high possibility of Se and nano-Si application toward the regulation of *A. annua* growth, biochemical characteristics (including essential oil and artemisinin) and mineral content.

Keywords: *Artemisia annua* L.; selenium; silicon nanoparticles; antioxidants; elemental composition

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1. Introduction

Among wormwood species, *Artemisia annua* L. attracts special attention due to its effectiveness against malaria, cancer [1], inflammation [2] and oxidative stresses [3,4]. This plant is rich with terpenoids; phenolic compounds, such as coumarins and flavonoids [5,6]; and lipopolysaccharides [2], and is considered to be the only plant source of artemisinin—the main sesquiterpene with a powerful antimalarial effect [7,8]. This sesquiterpene lactone

is very effective against chloroquine-resistant strains of *P. falciparum* malaria and also against various types of cancers and tumors; some viruses, including SARS-CoV-2; tuberculosis; and many other parasites, including *Schistosoma* sp., *Leishmania* sp., *Borrelia burgdorferi* and *Trypanosoma* sp. [9]. Artemisinin and its derivatives are released by *A. annua* within the glandular trichomes on the surface of the leaves, stems and flowers [8,10]. *A. annua* inhabits China, Australia, Argentina, Brazil, Bulgaria, France, Hungary, Italy, Spain, Romania, the United States, Mozambique, Altay and Buryatia [11,12], and is rarely found in European part of Russia, though, according to current and future climate scenarios, mid-latitudes in western and central Europe, southeastern Asia and North and South America are potentially suitable for *A. annua* growth and development [13]. However, to date, no information exists about the possibilities of plant production in the middle part of European Russia.

The wide biological activity of *A. annua* relates to a synergetic effect between the main components of its biologically active compounds, such as artemisinin, polyphenols and essential oil [14,15]. Nevertheless, to date, most of the investigations has been devoted to the improvement of artemisinin content, either via the utilization of different fertilizers [15], AMF [16] or various chemical elements supplementation: Mn and Zn [17], P [16], Na [18] and the ionic form of Si [19]. On the other hand, no attempts have been carried out regarding the evaluation of the Se supply efficiency with and without nano Si treatment. Such an approach may provide additional benefits in *A. annua* cultivation, as moderate doses of Se reportedly improve the plant yield and antioxidants accumulation [20] and modulate the hormonal status [21]. The latter phenomenon, along with the Si supply, results in an increase in trichomes development [22], i.e., the place of artemisinin production. Furthermore, along with the known beneficial effect of Se on the yield and nutritional quality of agricultural crops [20], the biofortification of *A. annua* may produce new challenges in human protection against oncological and viral diseases because Se was proven to be effective against cancer [20] and SARS-CoV-2 [23]. Therefore, the aim of the present study was the evaluation of the Se and nano-Si effect on the growth and biochemical characteristics of *A. annua* L. grown in the northern biosphere.

2. Material and Methods

2.1. Growing Conditions and Experimental Protocol

Research was conducted in 2020–2021, from April to September, at the experimental fields of the Federal Scientific Vegetable Center, Moscow region, Russia (55°39.51′ N, 37°12.23′ E), in a loam sod podzolic soil with the following initial soil characteristics: pH 6.2, 2.12% organic matter, 1.32 mg-eq/100 g hydrolytic acidity, 18.5 mg kg^{-1} mineral nitrogen, 21.3 mg kg^{-1} ammonium nitrogen, sum of absorbed bases as much as 93.6%, 402 mg kg^{-1} mobile phosphorous, 198 mg kg^{-1} exchangeable potassium, 1 mg kg^{-1} S, 10.95 mg kg^{-1} Ca, 2.05 mg kg^{-1} Zn, 0.86 mg kg^{-1} B, 220 µg kg^{-1} d.w. Se, 7.65 mg kg^{-1} Ni, 0.22 mg kg^{-1} Cd, 1.6 mg kg^{-1} As, 12.85 mg kg^{-1} Pb. The values of mean temperature and relative humidity during the vegetation period are presented in Table 1. The photoperiod duration (day length, hours) was 17.20–17.33 in June and 17.26–17.00 in July.

Table 1. Mean temperature and humidity in 2020 and 2021.

Month	2020		2021	
	Mean Temperature °C	Humidity %	Mean Temperature °C	Humidity %
May	13.5	66	13.5	66
June	21.0	73	17.2	69
July	23.81	74.0	20.1	72
August	17.8	72	17.8	75
September	11.9	80	11.9	80

Sowing was practiced on 1–3 May. In mid-June, plants were supplied with Se and/or Si using foliar treatment with sodium selenate solution (50 mg L^{-1}), colloidal solution of Si

nanoparticles (13 mg L^{-1}) and their joint application. Control plants were sprayed with water. The treatments were repeated on 10 August. Three replicates were used in this study, with each bed covering 1 m^2. To exclude the interference of other factors, no fertilizers were applied during the experiment. The results were expressed as means of the two-year data.

2.2. Colloidal Solution of Silicon Nanoparticles

Colloidal solution of Si nanoparticles was obtained by pulsed laser ablation in a liquid. The irradiation was achieved by using a pulsed nanosecond Nd:YAG laser with a wavelength λ = 1064 nm. The laser pulse length was 12 ns and pulse frequency 1 Hz. Rated energy in the pulse was 2.5 J. As a target, special-purity-grade single-crystalline silicon was sprayed. The target was immersed in a static glass cell with 250 mL of deionized water. The laser beam was focused on target inside the cell by lens. The target was irradiated for 30 min without stirring.

Determination of nano-Si concentration in the colloidal solutions was achieved using ICP-AES with ULTIMA 2 (Horiba Jobin Yvon, Longjumeau, France) spectrometer described in study by Golubkina et al. [24].

Nano-Si particles' size was measured by dynamic light scattering (DLS) method using Photocor Compact Z (Photocor, Tallinn, Estonia) laser analyzer (λ = 589 nm, laser rated-power output 32 mW).

The concentration of nano-Si in the colloidal solution was equal to 14 mg L^{-1}.

2.3. Sample Preparation

After harvesting in the middle of September and removal of soil particles from roots and stems, leaves, stems and roots of at least 10 plants were separated and homogenized. Fresh leaves homogenates were used for the determination of nitrates, ascorbic acid and photosynthetic pigments. The remaining parts of plants were dried at 70 °C to constant weight and homogenized, and the resulting powders were used for the determination of total polyphenols content (TP), total antioxidant activity (AOA), proline, malonic dialdehyde (MDA) and mineral composition.

2.4. Dry Matter

The dry matter was assessed gravimetrically by drying the samples in an oven at 70 °C until constant weight.

2.5. Nitrates

Nitrates were assessed using ion-selective electrode with ionomer Expert-001 (Econix Inc., Moscow, Russia) according to [24].

2.6. Elemental Composition

Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Na, Ni, P, Pb, Si, Sn, Sr, V and Zn contents in dried homogenized leaves were assessed using ICP-MS with quadruple mass-spectrometer Nexion 300D (Perkin Elmer Inc., Shelton, CT, USA) according to Golubkina et al. [24].

Trace levels of Hg and Sn in samples were not taken into account and, accordingly, they are not included in the tables.

2.7. Ascorbic Acid

The ascorbic acid content was determined by visual titration of leaf and stem extracts in 3% trichloracetic acid with sodium 2.6-dichlorophenol indophenolate solution (Tillman's reagent) [25]. Roots were not taken into consideration due to low ascorbic acid content.

2.8. Photosynthetic Pigments

Photosynthetic pigments were measured using 96% ethanolic extracts of *A. annua* leaves according to Lichtenthaler [26].

2.9. Total Polyphenols (TP)

Total polyphenols were determined in 70% ethanol extracts of dried samples using the Folin–Ciocalteu colorimetric method as previously described [27]. As an external standard, 0.02% gallic acid was used. The results were expressed as mg of gallic acid equivalent per g of dry weight (mg GAE g^{-1} d.w).

2.10. Antioxidant Activity (AOA)

The antioxidant activity of wormwood roots, stems and leaves was assessed on ethanolic extracts of dry samples, produced as described in Section 2.9 using a redox titration method [27]. The values were expressed in mg gallic acid equivalents (mg GAE g^{-1} d.w.).

2.11. Artemisinin

Artemisinin was determined using capillary electrophoresis according to [28] with a small modification. *A. annua* homogenate (4.00 ± 0.01 g with particle size less than 1 mm) was extracted with 25 mL of chloroform stirred for 48 h. The mixture was filtered and the solvent was removed on a rotary evaporator. The residue was dissolved in 10 mL of 70% ethanol, filtered again and used for the analysis. The study was carried out on a Capel-105M capillary electrophoresis system (OJSC Lumex-Marketing, Saint Petersburg, Russia) with quartz capillary parameters: diameter 75 µm, ratio of total capillary length to effective length 50/60 cm. The quartz capillary was preliminarily washed successively with distilled water, 1 M sodium hydroxide solution, distilled water, 1 M hydrochloric acid solution, distilled water and buffer solution.

The resulting solutions were filtered through a Vladipor membrane filter of the MFAS-B-4 type with disk diameter 25 mm and then centrifuged at 8000 rpm for 5 min. A 2 mL aliquot of ethanol extract was placed in a volumetric flask; the volume was adjusted to 25 mL with a 0.1 M solution of sodium hydroxide, and light absorption at 292 nm was determined using spectrophotometer UNICO (Dayton, NJ, USA).

Analysis conditions: sample injection was carried out hydro dynamically at 150 mbars; detection at a wavelength of 291 nm; voltage +20 kV; temperature in the capillary 20 °C; electrolyte: 10 mM borate buffer pH 9.2; analysis time—15 min.

Electrophoregrams were processed using the Elforan program (version 3.2.5; Lumex, Saint Petersburg). Artemisinin content was determined using standard curve built with 0.05–0.6 mg mL^{-1} artemisinin (Sigma-Aldrich, Buchs, Switzerland).

2.12. Proline

Proline concentration was determined according to [29] with slight modification. A total of 0.05 g of dry homogenized *A. annua* leaves was homogenized with 10 mL of 3% sulfur salicylic acid in a mortar. The mixture was filtered. One milliliter of the resulting filtrate, one milliliter of ninhydrin reagent and one milliliter of acetic acid were heated at 95 °C for 1 h. Proline concentration was evaluated using absorption value of the reaction mixture at 505 nm and a calibration curve with 5 different proline (Merck, Altoona, PA, USA) concentrations.

2.13. Malonic Dialdehyde

Approximately 100 mg of dried homogenized leaves was heated at 95 °C for 30 min with 5 mL of 0.5% tiobarbituric acid containing 10% of trichloroacetic acid. The resulting solutions after cooling were filtered, and absorption at 532 nm was determined. Calculation of MDA concentration was achieved using extinction value equal to 155 [30].

2.14. Selenium

Selenium was analyzed using the microfluorimetric method according to Alfthan [30]. The precision of the results was verified using a reference standard of Se-fortified chervil stem powder in each determination, with a Se concentration of 1865 µg·Kg^{-1} (Federal Scientific Vegetable Center, Moscow, Russia).

2.15. Essential Oil

Dry *A. annua* leaves/florets powder (50 g) was hydro-distilled in a Ginsberg-type apparatus for 2 h, and then percentage and yield of essential oils were calculated. The essential oils were dried over anhydrous sodium sulfate, stored in a dark glass vials and kept at 4 °C [31]. Composition of essential oil was investigated using gas-chromatograph "Chromatec-Kristall 5000.2" (Chromatec Inc., Ioshkar-Ola, Russia) with mass-spectrographic detector. Volatile components were separated with a capillary column CR-5ms (5% phenylmethyl-polysiloxane, 0.25 mm × 30 m; 0.25 µm film thicknesses). The temperature of injector and transfer line were set to 250 and 300, respectively. The oven was heated to 75 °C, subsequently 4.0 °C min^{-1}, and up to 240 °C; evaporator temperature −250 °C. The following conditions were adopted: split ratio 1:25, at flow 1.1 mL min^{-1}, with helium as carrier gas and injection volume of 1 mL of essential oil diluted in dichloromethane (1:300 *v*/*v*). The components of the essential oils were identified by comparison of their retention indices relative to (C_8-C_{30}) n-alkanes (Sigma-Aldrich, Buchs, Switzerland) and Supelco analytical standards (Sigma-Aldrich, St. Louis, MO, USA) and via comparison of their mass-spectra with those of NIST 14 mass spectra collection. (National Institute of Standards and Technologies, Gaithersburg, MD, USA).

2.16. Statistical Analysis

Data were processed by analysis of variance, and mean separations were performed through the Duncan's multiple range test, with reference to 0.05 probability level, using SPSS software version 21 (Armonk, NY, USA).

3. Results and Discussion

3.1. Morphological Characteristics

The growth stimulation effect of nano-Si, Se and, especially the joint application of nano-Si + Se, resulted in a significant increase in the leaves and stems weight compared to control plants (Table 2). All treatments significantly increased the leaves number by 40%, while the nano-Si + Se supply provided a 1.9 times increase in leaves length. On the contrary, no statistically significant changes in plant height were recorded.

Table 2. Morphological characteristics of *A. annua* plants subjected to sodium selenate and nano-Si (M ± SD).

	Control	Si	Se	Si + Se
Plant height (cm)	123 ± 15 a	131 ± 17 a	127 ± 16 a	127 ± 15 a
Leaf length (cm)	24 ± 4 b	25 ± 3 b	27 ± 4 b	45 ± 7 a
Number of leaves	14 ± 1 b	19 ± 2 a	19 ± 2 a	20 ± 3 a
Leaf weight (g)	26.03 ± 2.05 c	50.08 ± 5.10 b	52.63 ± 5.05 b	115.14 ± 9.71 a
Stem weight (g)	32.3 ± 2.78 c	41.94 ± 3.98 b	39.05 ± 3.04 b	76.79 ± 8.02 a
Leaf weight (% of plant weight)	44.6 ± 4.0 b	56.4 ± 4.2 a	57.4 ± 4.1 a	60.0 ± 5.0 a

Along each line, the values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

The duration of phenological phases was similar both for the control and Se/Si-treated plants. Despite the fact that the most intensive growth period of *A. annua* (15 July–15 August) was in accordance with the literature data [9], the cool September typical for the region investigated impeded plants to reach full maturity, so that, at harvest (mid-September), all plants were in the phenological phase between budding and the beginning of flowering.

A growth stimulation effect of Se applied at low doses has been reported in many plant species [20], and the present results are in agreement with these observations. A similar beneficial effect of low doses of nano-Si was manifested earlier only in chervil plants [24]. Nevertheless, these plants differ greatly in their response to the joint application of Se

and nano-Si. The data presented in Table 2 indicate that the growth stimulation effect of joint Se and nano-Si application on *A. annua* is twice higher than in the case of separate Se and Si utilization, whereas no differences were found in chervil in similar conditions [24]. The phenomenon may be connected with genetic peculiarities of the above plants and, in particular, with significant differences in the duration of the vegetation period.

3.2. Biochemical Characteristics

The effect of Si/Se foliar application on the dry matter, nitrates and total dissolved solids (TDS) content in *A. annua* plants demonstrated moderate changes in most cases. Indeed, according to Table 3 data, the applied treatments did not change the dry matter content in leaves, stems and roots of plants, with a mean roots:stems:leaves dry matter ratio equal to 1.23:1.05:1.00.

Table 3. Dry matter, nitrates and total dissolved solids (TDS) accumulation in *A. annua* under Se and/or Si supply (M ± SD).

Parameter	Plant Part	Control	Nano-Si	Se	Se + Nano-Si
Dry matter (%)	Leaves	13.0 ± 1.0 a	15.0 ± 1.2 a	13.7 ± 1.1 a	13.3 ± 1.0 a
	Stems	13.4 ± 1.1 a	15.6 ± 1.3 a	14.5 ± 1.3 a	14.3 ± 1.2 a
	Roots	16.6 ± 1.4 a	17.3 ± 1.5 a	17.5 ± 1.5 a	16.3 ± 1.4 a
Nitrates (mg g^{-1} d.w.)	Leaves	3.00 ± 0.25 a	3.04 ± 0.22 a	2.90 ± 0.21 a	2.76 ± 0.21 a
	Stems	5.38 ± 0.48 a	4.34 ± 0.35 b	3.93 ± 0.37 b	4.69 ± 0.37 ab
	Stems/leaves	1.79 ± 0.03 a	1.43 ± 0.03 b	1.36 ± 0.02 b	1.70 ± 0.04 a
TDS (mg g^{-1} d.w.)	Leaves	67.7 ± 6.1 a	67.2 ± 6.1 a	73.1 ± 6.9 a	67.1 ± 6.1 a
	Stems	127.7 ± 11.1 a	97.0 ± 8.8 b	89.4 ± 8.2 b	105.5 ± 9.0 ab
	Stems/leaves	1.89 ± 0.06 a	1.44 ± 0.06 bc	1.22 ± 0.05 c	1.57 ± 0.04 b

TDS: total dissolved solids. Along each line, the values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

According to Table 3 data, neither the separate nor joint application of Se and nano-Si affect nitrate and total dissolved solids (TDS) levels in *A. annua* leaves ($p > 0.05$). On the contrary, the same parameters when referring to stems showed statistically significant decreased values under a separate Se and Si supply, though, at a small magnitude. Apparently, the effect of the sodium selenate foliar supply on the nitrates and TDS content in plant leaves is species-specific, as a 1.5-fold leaf TDS increase and 5-fold nitrate decrease have been reported previously in Indian mustard due to selenate biofortification [32].

3.3. Photosynthetic Pigments

Selenium is known to participate in chlorophyll biosynthesis [33], the phenomenon indicated also on *A. annua* (Table 4). In the latter case, we recorded a 20% increase in the chlorophyll a content and a tendency for the values to increase in the case of chlorophyll b and carotene due to Se application. On the other hand, nano-Si affected *A. annua* differently, because it significantly decreased the chlorophyll accumulation by 28%, but did not influence the carotene content. The latter phenomenon is opposite to that described in chervil plants treated with similar doses of nano-Si and Se [24] and in other Se-fortified plant species [34,35]. A more valuable carotene improvement was recorded for the joint application of Se and nano-Si, providing a 23% increase in the parameter. These facts prove the significance of the effect of genetic factors on the plant's response to the Se and Si supply.

Table 4. Photosynthetic pigment content in *A. annua* leaves as affected by nano-Si and/or sodium selenate (M ± SD).

Parameter	Control	Nano-Si	Se	Se + Nano-Si
Chl a (mg g^{-1} f.w.)	1.32 ± 0.11 b	1.01 ± 0.09 c	1.66 ± 0.13 a	1.45 ± 0.12 ab
Chl b (mg g^{-1} f.w.)	3.48 ± 0.30 a	2.73 ± 0.21 b	3.89 ± 0.32 a	3.61 ± 0.31 a
Total chl (mg g^{-1} f.w.)	4.80 ± 0.42 a	3.74 ± 0.33 b	5.55 ± 0.50 a	5.06 ± 0.45 a
Carotene (mg g^{-1} f.w.)	0.56 ± 0.04 b	0.49 ± 0.03 b	0.68 ± 0.06 a	0.70 ± 0.06 a
Chl b/Chl a	2.64 ± 0.02 a	2.70 ± 0.02 a	2.34 ± 0.03 a	2.49 ± 0.02 a
Carotene /total chl (%)	11.9 ± 0.01 a	13.1 ± 0.01 a	12.3 ± 0.01 a	13.8 ± 0.02 a

Chl: chlorophyll. Along each line, values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

3.4. Antioxidant Status

The improvement of the plant antioxidant status due to nano-Si application is one of the mechanisms for plant protection against oxidant stresses [34], though extremely little information is available about the nano-Si effect on the plants' antioxidant status in ordinary conditions of vegetation without significant stress factors. In case of *A. annua*, both the separate and joint application of Se and Si significantly improved ascorbic acid (AA) accumulation (Table 5). In this respect, in similar conditions, these elements did not cause any effect on ascorbic acid accumulation in chervil [24].

Table 5. Antioxidant status of *A. annua* L. at flowering stage (M ± SD).

	Tissue	Control	Si	Se	Se + Si
AA (mg 100 g^{-1} f.w.)	Leaves	74.2 ± 7.1 b	106.0 ± 8.5 a	90.6 ± 8.5 a	106.3 ± 8.2 a
AOA (mg GAE g^{-1} d.w.)	Leaves	48.7 ± 4.2 b	75.1 ± 7.0 a	75.2 ± 7.1 a	52.7 ± 5.1 b
	Stems	12.2 ± 1.2 b	17.4 ± 1.6 a	16.6 ± 1.6 a	15.7 ± 1.5 a
	Roots	7.1 ± 0.7 a	6.9 ± 0.6 a	8.5 ± 0.7 a	7.5 ± 0.7 a
TP (mg GAE g^{-1} d.w.)	Leaves	23.2 ± 2.1 a	24.7 ± 2.2 a	26.6 ± 2.3 a	23.3 ± 2.1 a
	Stems	10.1 ± 1.0 a	12.4 ± 1.2 a	12.2 ± 1.1 a	12.0 ± 1.1 a
	Roots	5.7 ± 0.5 b	5.6 ± 0.5 b	7.3 ± 0.7 a	6.0 ± 0.6 ab
MDA (mM g^{-1} d.w.)	Leaves	0.38 ± 0.04 a	0.31 ± 0.03 a	0.35 ± 0.03 a	0.36 ± 0.03 a
Proline (mg g^{-1} d.w.)	Leaves	8.14 ± 0.81 ab	8.21 ± 0.81 ab	7.67 ± 0.78 b	9.41 ± 0.91 a
Artemisinin (mg kg^{-1} d.w.)	Leaves	3.85 ± 0.17 b	3.94 ± 0.11 b	3.68 ± 0.14 b	5.42 ± 0.25 a

AA: ascorbic acid; AOA: total antioxidant activity; TP: total phenolics; MDA: malonic dialdehyde. Along each line, values with the same letters do not differ significantly according to Duncan's test at $p < 0.05$.

A positive effect of Si/Se application on ascorbic acid accumulation seems to be one of the most significant effects for the *A. annua* antioxidant status, with the mean AA increasing by a factor of 1.36. Regarding the total antioxidant activity (AOA), a statistically significant improvement of this parameter was registered for nano-Si (1.52) and Se (1.54 times) treatments. On the contrary, the joint application of Se + nano-Si did not change AOA and total phenolics (TP) levels in leaves. The mean AOA leaves/stems/roots ratio reached 6.86:1.72:1 for control plants; 10.88:2.21:1- for nano-Si; 8.85:1.95:1 for Se; and 7.03:2.09:1 for the joint application of Se and nano-Si. Accordingly, the distribution of phenolics (TP) between leaves, stems and roots reached 4.07:1.77:1 (control); 4.41:2.21:1 (nano-Si); 3:1.67:1 (Se); and 3.88:2:1 (Se + nano-Si).

The close relationship between AOA and TP parameters (Figure 1) indicates a non-linear character of the curve with a higher variability of AOA in leaves, indicating that the leaves AOA is affected not only by the TP content but also by other antioxidants

and essential oil that are particularly known to accumulate predominantly in leaves and florets [36].

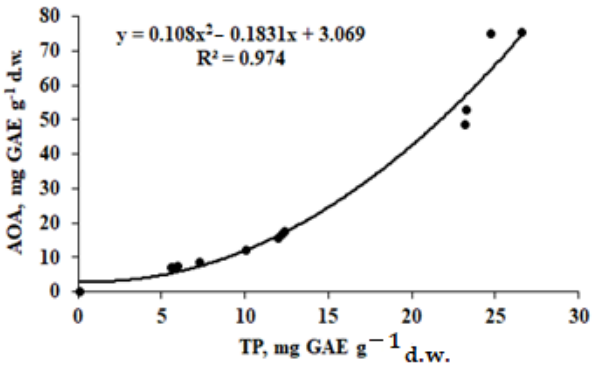

Figure 1. Correlation between AOA and TP in *A. annua* plants (r = 0.987, $p < 0.001$).

At the same time, the non-statistically-significant changes in the proline and malonic dialdehyde (MDA) content in *A. annua* treated with Se and/or nano-Si indicate a low level of oxidant stress during vegetation. The exception is the high concentration of proline in plant leaves under the joint application of Se and nano-Si, which suggests an induction of plant tolerance. According to [37], high concentrations (150 mg L^{-1}) of nano-Si mitigates lipid peroxidation and boosts osmoprotection. Taking into account the low concentrations of Se and especially Si in the present investigation, these factors supposedly provided only a low effect on MDA and proline changes.

3.5. Essential Oil Accumulation

The essential oil chemical profile is generally influenced by the harvesting season, fertilizer and the pH of soils, the choice and stage of drying conditions, the geographic location, chemotype or subspecies, and the choice of the part plant or genotype or extraction method.

It is evident that the geographic location greatly influences the type and concentration of the main constituents. Other factors that determine the chemical composition and thus the biological activity are the chemical and biological treatment.

Eucalyptol, artemisia ketone, camphor and germacrene D are known to be the main components of *A. annua* essential oil, with the concentration being in the range of 4.8–31.5%, 2.2–68.5%, 1.9–48.0%, and 10.9–21.2%, respectively [38].

All of these chemicals demonstrate high antioxidant, anti-inflammatory, anti-viral, anti-bacterial and analgesic properties [39,40].

Essential oil accumulation is known to be directly connected with the antioxidant activity of Artemisia leaves [41]. Up to date, rather scant data are available on the effect of nano-Si supplementation on essential oil accumulation in aromatic plants: lemongrass *Cymbopogon flexuosus* [37], *Mentha piperita* [42] and coriander *Coriandrum Sativum* L. [43] (concentrations applied were within 42–150 mg L^{-1}). In addition, separate data indicate the possibility of essential oil synthesis stimulation as a result of sodium selenate foliar application [44].

In general, biological effects of *A. annua* essential oil are the result of a synergism of all molecules contained in an essential oil, even if it is possible that the activity of the main components is modulated by other minor molecules, but the activity of the isolated constituents is also remarkable [38].

The essential oil content and number of constituents in *A. annua* plants greatly depend on the plant ontogenetic conditions [45,46]. Significant changes in the essential oil yield and its components number as a result of the single and joint application of nano-Si and Se indicate phenophase shifts, which are directly connected with a significant increase in the biomass of Se/Si-treated plants (Tables 2 and 6, Figure 2). Nano-Si foliar application seems to be the greatest effector of these parameters. Table 6 and Figure 2 data indicate that,

under these conditions, the number of essential oil components decreased significantly by 1.6 times and was accompanied by a significant increase in eucalyptol by 2.36 times, and in artemisia ketone by 3.78 times, whereas the germacrene D content decreased by 1.84 times. Whether the phenomenon relates to the decreased accumulation of photosynthetic pigments in plants treated with nano-Si needs further investigation.

Table 6. Yield and composition of *A. annua* essential oil under Se and/or Si supply (M ± SD).

Parameter	Control	Si	Se	Si + Se
Essential oil yield (%)	0.20 ± 0.01 a	0.15 ± 0.01 b	0.15 ± 0.01 b	0.10 ± 0.01 c
Number of components	81 ± 2 a	50 ± 1 c	71 ± 1 b	72 ± 2 b
The Main Oil Components, %				
Eucalyptol	3.32 ± 0.30 b	7.85 ± 0.75 a	1.83 ± 0.16 c	3.06 ± 0.29 b
Artemisia ketone	6.28 ± 0.63 c	23.73 ± 2.33 a	4.39 ± 0.41 d	8.36 ± 0.82 b
Camphor	32.83 ± 2.90 a	34.35 ± 3.04 a	23.02 ± 2.04 b	21.61 ± 2.10 b
Germacrene D	9.25 ± 0.91 b	5.03 ± 0.50 c	11.77 ± 1.13 a	10.17 ± 1.02 ab

Along each line, values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

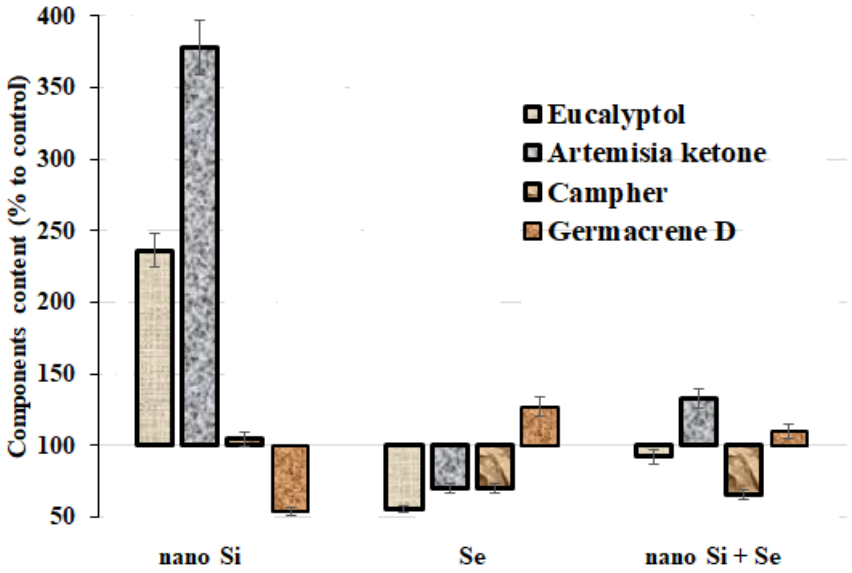

Figure 2. Effect of separate and joint application of nano-Si and Se on essential oil content in *Artemisia annua*, grown in Moscow region.

The separate and joint nano-Si of Se application significantly decreased the camphor content by 1.4–1.5 times. Besides camphor, eucalyptol and artemisia ketone demonstrated a significant decrease under a single supply of sodium selenate.

A significant decrease in the number of oxidized forms of essential oil due to the Se supply may be connected with antioxidant properties of this element (the examples are camphor, pinocarvon, artemisia ketone and eucalyptol). On the contrary, Se seems to stimulate the production of carbohydrates, such as β-Selinene, humulene, (E)- β-famesene, β-caryophyllene, germacrene D and camphene (see supplement, Table S1). However, it should be indicated that there is at least one exception: caryophyllene and caryophyllene oxide both demonstrated a significantly increased concentration as a result of Se and Se + nano-Si treatment.

Several studies have permitted the conclusion that the *A. annua* crop could be harvested much before the onset of flowering in order to obtain high yields of artemisinin, and the crop must be allowed to attain maturity to obtain high yields of the essential oil [8,10]. Finally, the planting date and harvest time can influence the maximum concentration of the

produced essential oil [10]. In the present work, at harvesting, *A. annua* plants were only at the stage of blooming and the beginning of flowering, which is not optimal for essential oil production.

3.6. Artemisinin Accumulation

In these conditions, a low content of artemisinin was recorded in *A. annua* leaves/florets (Figure 3) compared to plants grown in a warm climate. Usually, the artemisinin content in *A. annua* has a wide concentration range (0.01–0.45%) [9] depending on the habitat, climate [47,48], soil and plant characteristics [49–51], time of sowing, planting and harvesting [52] and plant genetic potential [9,13]. A lack of significant stress factors, which are known to stimulate the production of artemisinin [53–56], relatively low temperature and high humidity reduced artemisinin production in conditions of the Moscow region. Despite nano-Si being known to stimulate glandular trichomes development (the place of artemisinin biosynthesis) [49], no beneficial effect of Si nanoparticles was recorded in the present work. The latter fact may be attributed to the low concentration of Si nanoparticles, which was 10 times lower than that described in the literature [9]. Nevertheless, a significant beneficial effect on artemisinin accumulation was revealed under the joint application of Se and nano-Si (Figure 4).

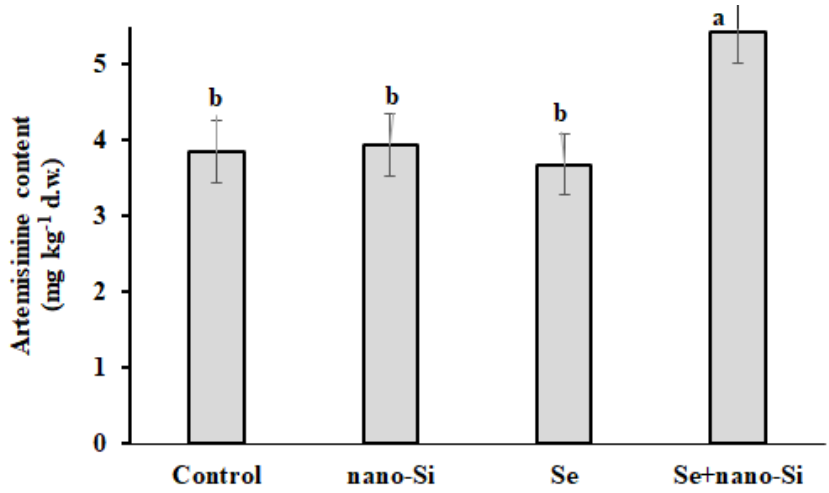

Figure 3. Effect of nano-Si and sodium selenate on artemisinin content. nts). Values with the same letter do not differ statistically according to Duncan test at $p < 0.05$.

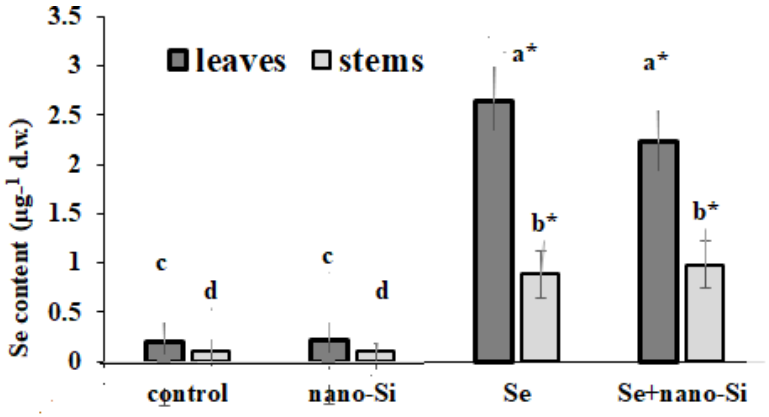

Figure 4. Effect of Se and nano-Si on leaves/stems Se distribution (*: values have been halved). Values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

3.7. Mineral Composition

3.7.1. Se Accumulation

According to literature data, nano-Si does not significantly affect Se accumulation in plants [24]. The results of the present research regarding *A. annua* Se biofortification are in accordance with bibliographic data indicating significantly higher Se concentrations in leaves compared to stems (Figure 4). Furthermore, the differences between Se levels in plants treated separately or jointly with Se and nano-Si were not statistically significant.

From a practical point of view, Se concentrations in *A. annua* plants arising from Se biofortification may provide up to 11 µg of Se per 5 g of dry leaves weight, which corresponds to 16% of the adequate Se consumption level in the case of plant utilization as a spice. In the case of *A. annua* tea application, 50 mL of water extract (1:1) [57] provides approximately 75% (52.4 µg Se) of the daily adequate Se consumption level, taking into account that approximately 50% of Se in the plant is represented by water-soluble forms of this element.

3.7.2. Other Elements

The singular and joint plant supplementation with Se and Si greatly changed macro- and microelements accumulation, causing either a redistribution of elements between leaves/stems or a decrease/rise in the accumulation levels.

Data presented in Tables 7–9 show a specific peculiarity of *A. annua* to significantly decrease the levels of Ca and Mg as a result of the separate and joint Se/Si supply, as well as a decrease in Ca in the case of Se and Se + Si supplementation. Despite the essentiality of these macro-elements in plants, no signs of any growth inhibition and photosynthetic pigments level decrease were recorded. On the contrary, Se solely or in combination with Si stimulated the biosynthesis of chlorophyll and carotene in A. *annua* leaves and did not cause any significant changes in proline and MDA levels, which are indicators of the environmental stress degree (Tables 4 and 5). The statistically significant decrease in chlorophyll concentration was recorded only under the Si supply, but, in these conditions, only slight changes in the mineral composition of plants were recorded (Tables 7–9).

Table 7. Macroelements in *A. annua* leaves and stems as affected by nano-Si, sodium selenate and nano Si + Se (M ± SD; mg kg^{-1} d.w.).

Element	Control	Si	Se	Se + Si
		Leaves		
Ca	10,778 ± 1057 a	8678 ± 812 b	6789 ± 628 c	8253 ± 800 bc
K	75,681 ± 7405 a	71,512 ± 6998 a	45,027 ± 4220 b	52,833 ± 5179 b
Mg	3718 ± 357 a	2810 ± 277 b	2743 ± 268 b	2679 ± 260 b
Na	514 ± 50 b	438 ± 44 b	491 ± 48 bc	677 ± 67 a
P	6177 ± 602 a	5172 ± 511 ab	5400 ± 535 a	5150 ± 506 ab
		Stems		
Ca	9328 ± 901 a	7006 ± 688 b	6821 ± 632 b	8044 ± 795 a
K	78,648 ± 7004 a	72,945 ± 6987 a	69,363 ± 6123 a	75,420 ± 7224 a
Mg	1512 ± 150 a	1495 ± 145 a	1129 ± 110 b	1327 ± 133 a
Na	312 ± 29 a	314 ± 30 a	381 ± 34 a	315 ± 30 a
P	4799 ± 456 a	3391 ± 329 b	4571 ± 446 a	3817 ± 377 b

Along each line, values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

Table 8. Heavy metals, As and Al in *A. annua* leaves as affected by nano-Si, sodium selenate and nano Si + Se (M ± SD; mg kg^{-1} d.w.).

Element	Control	Si	Se	Se + Si
		Leaves		
Al	61.3 ± 6.1 b	57.2 ± 5.7 b	128.0 ± 12.8 a	41.1 ± 4.0 c
As	0.15 ± 0.01 a	0.15 ± 0.01 a	0.09 ± 0.01 b	0.08 ± 0.01 b
Cd	0.46 ± 0.04 a	0.29 ± 0.03 b	0.25 ± 0.02 b	0.20 ± 0.02 c
Cr	0.42 ± 0.04 b	0.46 ± 0.05 b	0.62 ± 0.06 a	0.32 ± 0.03 c
Ni	1.49 ± 0.15 a	1.17 ± 0.11 b	1.45 ± 0.14 a	0.91 ± 0.09 b
Pb	0.66 ± 0.06 a	0.78 ± 0.07 a	0.39 ± 0.04 c	0.53 ± 0.05 b
Sr	13.10 ± 1.30 a	11.8 ± 1.10 ab	9.68 ± 0.92 c	10.82 ± 1.03 bc
V	0.68 ± 0.06 a	0.75 ± 0.07 a	0.77 ± 0.07 a	0.42 ± 0.04 b
		Stems		
Al	104.0 ± 10.4 a	55.3 ± 5.5 b	47.4 ± 4.7 b	45.1 ± 4.5 b
As	0.06 ± 0.01 a	0.04 ± 0.01 b	0.03 ± 0.001 c	0.03 ± 0.001 c
Cd	0.82 ± 0.08 a	0.87 ± 0.08 a	0.87 ± 0.09 a	0.73 ± 0.07 a
Cr	1.45 ± 0.14 b	2.35 ± 0.22 a	1.44 ± 0.14 b	2.99 ± 0.30 a
Ni	1.62 ± 0.16 a	1.74 ± 0.17 a	1.23 ± 0.12 b	1.59 ± 0.15 ab
Pb	3.36 ± 0.33 a	1.21 ± 0.12 b	0.83 ± 0.08 c	0.98 ± 0.10 bc
Sr	28.17 ± 2.80 a	23.53 ± 2.35 a	25.94 ± 2.45 a	25.91 ± 2.51 a
V	0.32 ± 0.03 a	0.23 ± 0.02 b	0.14 ± 0.01 c	0.15 ± 0.01 c

Along each line, values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

Table 9. Microelements content in *A. annua* leaves as affected by nano-Si, sodium selenate and nano Si + Se (M ± SD; mg kg^{-1} d.w.).

Element	Control	Si	Se	Se + Si
		Leaves		
B	54.81 ± 5.46 a	37.77 ± 3.80 b	31.95 ± 3.20 bc	29.69 ± 3.00 c
Co	0.12 ± 0.01 de	0.14 ± 0.01 cd	0.10 ± 0.01 e	0.06 ± 0.01 f
Cu	20.17 ± 2.02 a	19.76 ± 2.00 a	16.41 ± 1.62 b	16.18 ± 1.60 b
Fe	277.0 ± 27.5 a	240.0 ± 24.1 ab	216.0 ± 21.3 b	148.0 ± 14.7 c
Li	0.06 ± 0.01 d	0.16 ± 0.02 a	0.14 ± 0.01 b	0.08 ± 0.01 c
Mn	40.60 ± 4.03 a	30.01 ± 3.00 b	25.72 ± 2.55 c	22.19 ± 2.19 c
Mo	1.22 ± 0.12 b	2.18 ± 0.22 a	1.18 ± 0.11 b	1.26 ± 0.12 b
Si	10.39 ± 1.04 a	10.11 ± 1.00 a	10.41 ± 1.02 a	9.32 ± 0.92 a
Zn	111.0 ± 11.2 a	114.0 ± 11.3 a	83.1 ± 8.3 b	68.1 ± 6.8 c
		Stems		
B	<0.021 a	<0.021 a	<0.021 a	<0.021 a
Co	0.23 ± 0.02 a	0.15 ± 0.01 bc	0.14 ± 0.01 c	0.17 ± 0.02 b
Cu	11.34 ± 1.13 a	9.07 ± 0.90 a	11.03 ± 0.11	9.38 ± 0.09 a
Fe	170.0 ± 17.0 a	103.0 ± 10.1 b	77.7 ± 7.7 c	87.8± 8.7 bc
Li	0.15 ± 0.01 a	0.15 ± 0.02 a	0.09 ± 0.01 b	0.10 ± 0.01 b
Mn	24.09 ± 2.40 a	21.84 ± 2.12 a	17.45 ± 1.73 b	16.73 ± 1.64 b
Mo	0.86 ± 0.08 b	1.21 ± 0.12 a	0.99 ± 0.10 ab	1.04 ± 0.10 ab
Si	9.14 ± 0.91 a	8.04 ± 0.80 a	6.64 ± 0.65 b	7.85 ± 0.78 ab
Zn	96.6 ± 9.6 b	107.0 ± 10.0 b	106.0 ± 10.0 b	159.0 ± 15.0 a

Along each line, values with the same letters do not differ statistically according to Duncan's test at $p < 0.05$.

Earlier investigations on spinach [21] confirmed the possibility of this phenomenon. Taking into account that a significant decrease in Ca, K and Mg under the selenite and selenate supply was recorded only in male spinach forms, it may be supposed that a similar phenomenon in *A. annua* plants may be connected with hormonal regulation, though this topic needs further investigation.

Furthermore, Se treatment also significantly reduced the accumulation of B, Cu, Fe, Mn and Zn in *A. annua* leaves. The suggestion of Łukaszewicz et al. [58] that the similar

microelement (Fe, Zn, Mn, B) decrease in pea due to Se^{+6}/Se^{+4} application is connected with dehydration associated with the membrane damage of roots does not seem applicable to *A. annua* plants, as no growth inhibition or photosynthetic pigment decrease was recorded. The effect is certainly species-dependent, as no significant differences were recorded in chervil grown and treated in similar way [24].

The matter of the Se/Si effect on essential macro- and trace elements accumulation has been poorly discussed in literature, and the experimental data often demonstrate controversial results. Indeed, the foliar biofortification of *A. cepa* with sodium selenate in the Chechen republic conditions did not cause any statistically significant changes in macroelements, whereas, among microelements, the B content increased as a result of the Se supply [59]. Maize seedlings obtained in hydroponics showed a Ca and P increase and significant decrease in K content as a result of the selenite supply [60–63]. No significant changes in macro and essential microelements accumulation were recorded in chickpea treated with sodium selenate [61]. In chervil, the Se supply decreased only the content of Na [24].

Regarding heavy metals, the singular or joint application of Si and Se resulted in a significant decrease in leaf As, Ca, Pb and Sr content, whereas the joint application of Se and Si caused the addition of other heavy metals to this list: Cr, Ni and V. These results are in agreement with the known antagonistic relationship between heavy metals and Se [62,63] and the protective effect of nano-Si against the former [64,65].

In the present work, a valuable redistribution of Li, Zn and Al between leaves and stems of *A. annua* as a result of Se/Si treatment was revealed for the first time (Tables 8 and 9). Indeed, a two-fold increase in leaf Al and Li levels as a result of the foliar Se^{+6} supply was accompanied by a corresponding significant decrease in stem Al/Li concentration. The joint application of Se and Si resulted in a significant decrease in the Zn level in the leaves and its increase in stems.

In general, the leaves/stems distribution of elements in *A. annua* plants indicates the predominant accumulation of Mg, P, V, B, Cu, Fe, Mn, Mo and Se in leaves and Al, Cd, Cr, Pb and Sr in stems (Tables 6–8), where only trace levels of B were found.

In the present investigation, low doses of nano-Si were not sufficient to elicit a statistically significant increase in Si concentration, both in the stems and leaves of *A. annua*, characterized by a high biomass reflecting significant biological dilution. In this respect, it should be highlighted that the beneficial effect of a low dose of nano-Si on the plant yield and antioxidant status is associated with a new phenomenon of nanoparticles application.

4. Conclusions

The results of the present experiment indicate typical peculiarities of *A. annua* Se biofortification under a nano-Si supply: a growth stimulation effect and increase in the antioxidant status of plants, with a significant reduction in the content of essential oil and number of its components, as well as profound qualitative and quantitative changes in the oil composition. Neither Se nor nano-Si in a singular application changed the artemisinin levels, whereas the joint supply of Se and nano-Si to plants significantly increased the artemisinin level by 1.4 times. The effect of Al, Li and Zn redistribution between leaves and stems, due to Se/Si supplementation and the anomalous decrease in Ca, Mg, K, B, Cu, Fe, Mn and Zn levels in leaves, as a result of the Se treatment not accompanied by growth inhibition or a significant decrease in the photosynthetic pigments content, needs further investigations in order to reveal the mechanism of the phenomenon.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae8070597/s1. Table S1: Effect of Se and nano-Si supply on *A. annua* essential oil composition.

Author Contributions: Experiment conceptualization: N.G., L.L., L.S. and G.C.; silicon nanoparticles production and characteristics provision: M.F. and A.A.; field experiment management and determinations: N.G. and L.L.; biochemical analysis: N.G. and L.S.; essential oil determination and

components identification: L.L. and O.S.; artemisinin determination: D.K. and E.G.; data statistical processing: N.G., L.S., A.S. and G.C.; data interpretation and draft and final-version manuscript writing: N.G., L.L, O.S., L.S., M.F., D.K., E.G., A.S. and G.C. All authors have read and agreed to the published version of the manuscript.

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