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Comparative evaluation of *Raphanus sativus* var. *lobo* defense efficiency against *Contarinia nasturtii* using foliar application of sodium selenate, ionic silicon form and garlic extract

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ABSTRACT

Relevance. *Contarinia nasturtii* Keiffer is one of the most dangerous insect pests of Brassicaceae plants dramatically affecting plant yield and seed productivity.

Material and Methods. The effect of single foliar application of sodium selenate, ionic form of silicon (Siliplant fertilizer) and garlic extract on the efficiency of *Raphanus sativus* lobo defense against *Contarinia nasturtii* infestation was evaluated in condition of greenhouse. Concentrations of the applied reagents were: sodium selenate 26.4 mM; Siliplant – 1 ml/l; garlic extract – 8 g/l. Seed productivity and *C. nasturtii* infestation levels under Se, Si and garlic extract administration under infestation were determined along with the analysis of plant antioxidant status and sugar content.

Results. Foliar application of Siliplant singular or in combination with Se provided the highest seed productivity and mass of 1000 seeds. Plants treated with Si, garlic extract and Si+Se demonstrated lack of *C. nasturtii* infestation only a week after the preparation supply. Contrary, selenate provided less pronounced defense. Among biochemical parameters tested the values of the total dissolved solids were the highest in plants treated with Se and Se+Si. Changes in the intensity of plant defense due to garlic, Se and Si supply were reflected in the increase of the total phenolic content compared to the values typical for control plants and in a significant accumulation increase of disaccharides, participating in tissue recovery after herbivory attack. Silicon application also provided an increase of selenium accumulation by lobo leaves.

KEYWORDS:

Raphanus sativus var. *lobo*, *Contarinia nasturtii*, selenium, silicon, garlic extracts, protection against pests

Сравнительная оценка эффективности защиты *Raphanus sativus* var. *lobo* от крестоцветной галлицы (*Contarinia nasturtii*), используя внекорневое внесение селената натрия, ионной формы кремния и экстракта чеснока

РЕЗЮМЕ

Актуальность. Крестоцветная галлица *Contarinia nasturtii* Keiffer – один из наиболее опасных вредителей растений семейства Brassicaceae, снижающих семенную продуктивность растений.

Материал и методика. Исследована эффективность однократного внекорневого внесения селената натрия, ионной формы кремния (удобрение Силиплант) и экстракта чеснока на эффективность защиты растений лобо *Raphanus sativus* lobo от *Contarinia nasturtii* в условиях закрытого грунта. Используемые концентрации: селена 26.4 mM раствор, Силипланта – 1 мл/л, экстракта чеснока – 8 г/л. Проводили оценку семенной продуктивности и уровней инфицирования растений крестоцветной галлицей, а также определяли показатели антиоксидантного статуса растений и содержания сахаров.

Результаты. Использование Силипланта без и совместно с селенатом натрия обеспечивало наибольшую семенную продуктивность растений лобо и наибольшую массу 1000 семян. Обработка семенных растений кремнием, экстрактом чеснока и совместное применение кремния и селена обеспечивали 100% защиту растений от *C. nasturtii* уже через неделю после обработки. Напротив, применение селената натрия обеспечивало лишь частичную защиту. Среди исследованных биохимических показателей уровень водорастворимых соединений (TDS) был наибольшим у растений, обработанных селеном без и на фоне использования кремния. Изменения в интенсивности защиты растений от крестоцветной галлицы благодаря использованию экстракта чеснока и растворов селената натрия и Силипланта отражались в возрастании уровней накопления полифенолов в листьях по сравнению с данными для контрольных полностью инфицированных растений и в значительном возрастании накопления дисахаридов, участвующих в восстановлении тканей после атаки вредных насекомых. Установлено также, что кремний стимулирует аккумуляцию селена листьями растений как без, так и при обработке растений селенатом натрия.

КЛЮЧЕВЫЕ СЛОВА:

Raphanus sativus var. *lobo*, *Contarinia nasturtii*, селен, кремний, экстракт чеснока, защита от вредных насекомых

Introduction

One of the most dangerous insect pests of *Brassicaceae* plants is *Contarinia nasturtii* Keiffer dramatically affecting yield and seed productivity of broccoli, cauliflower, cabbage, Brussels sprouts, turnip, radish, etc. It is widely distributed in Europe, Scandinavia, Mediterranean countries, Turkey and western Russia [1]. *Contarinia nasturtii* lays eggs in inflorescences, causing severe damage to seed yield, loba seeds in particular, preventing seed set. Literature data indicate that seed yield losses caused by *C. nasturtii* may reach more than 85% [2].

The main methods of *C. nasturtii* control include synthetic insecticides application (such as neonicotinoids) and crop rotation [3-6]. On the other hand, numerous investigations indicate high prospects of garlic extract utilization [7] and application of selenium (Se) and silicon (Si) preparations [8-10] in insect pest control. Thus, the silicon application provides reduced oviposition, feeding and digestibility of insects. Selenium in relatively high concentrations results in low pest reproduction, increased mortality and reduced pest longevity. Aqueous garlic extract is a plant biostimulant, improves crop quality and provides the defense responses of receiver plants [11].

The attractiveness of Se, Si and garlic extract application in protection of plants is also connected with a well-known fact of the growth-stimulation effect, increase in plant immunity and tolerance to various biotic and abiotic stresses [8]. In this respect, the aim of the present investigation was evaluation of the efficiency of *Raphanus sativus* var. lobo protection against *Contarinia nasturtii* using foliar application of sodium selenate, potassium silicate (Siliplant fertilizer) and garlic extract. Margelan radish (*Raphanus sativus* var. lobo) is originated from China and is grown in most countries of the world. Its popularity is connected with high nutritional value, pleasant taste and low content of radish oil. *C. nasturtii* infestation of plants is a serious problem of Lobo seed production.

2. Material and Methods

2.1. Growing Conditions and Experimental Protocol

Planting of seed plants of Loba cv Krasavitsa Podmoskovya was carried out in a greenhouse on 20 April with spacing of 30 cm between the plants along the rows which were 70 cm apart. Before planting, milling was carried out to a depth of 20 cm. Regular watering (three times per week), double $N_{15}P_{15}K_{15}$ fertilization at a rate of 40 kg/Ha during the peduncle formation phase were applied. During the period of the peduncle formation – the beginning of budding/flowering, with the primary signs of the manifestation of flower infection foliar treatments with selenium, silicon and garlic extract preparations began (15 June). Treatments were carried out three times with an interval of 4-7 days in the evening hours, after watering, when the temperature in closed ground dropped to 20...25 °C. The experimental treatments, applied to Loba were carried out according to the following scheme: (1) control (water foliar spray), (2) sodium selenate solution, 26.4 mM (50 mg L⁻¹), (3) Siliplant treatment, 1 ml/L; (4) sodium selenate + Siliplant (the same concentrations), and (5) garlic extract. Garlic extract was prepared via the homogenization and extraction of fresh garlic bulbs with distilled water (8 g:1000 mL) and the filtration of a mixture. A split plot design was used for the treatment distribution, with three replicates and each experimental unit covering 10 m².

2.2. Sample Preparation

2.2.1. Seeds

On the 10 of September after full ripening, number of pods, number of seeds per pod, mass of 1000 seeds were measured.

2.2.2. Samples for biochemical analysis

A small amount (3-5) of fresh leaves from each plant were gathered at the stage of seed formation (15 August), homogenized and used for the determination of nitrates and the ascorbic acid content. The remaining parts of plant homogenates were dried at 70 °C to constant weight and homogenized for obtaining fine powders. The latter were used for the determination of the total antioxidant activity (AOA), total polyphenols (TP), sugar and Se content.

2.3. Dry Matter

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

2.4. Selenium

Selenium content was analyzed using the fluorimetric method previously described for tissues and biological fluids [12]. Dried homogenized samples were digested via heating with a mixture of nitric and perchloric acids, subsequent reduction of selenate (Se⁺⁶) to selenite (Se⁺⁴) with a solution of 6 N HCl, and the formation of a complex between Se⁺⁴ and 2,3-diaminonaphthalene. Se concentration was calculated by recording the piazoselenol fluorescence value in hexane at 519 nm λ emission and 376 nm λ excitation. Each determination was performed in triplicate. The precision of the results was verified using the mitsuba reference standard of Se-fortified stem powder in each determination, with a Se concentration of 1865 μ g kg⁻¹ (Federal Scientific Vegetable Center).

2.5. Total Polyphenols (TP)

Total polyphenols were determined in 70% ethanol extracts of samples using the Folin–Ciocblteu colorimetric method as previously described [13]. Half a gram of dry homogenates was extracted with 20 mL of 70% ethanol/water at 80°C for 1 h. The mixture was cooled down and quantitatively transferred to a volumetric flask, and the volume was adjusted to 25 mL. The latter mixture was filtered through filter paper, and 1 mL of the resulting solution was transferred to a 25 mL volumetric flask, to which 2.5 mL of saturated Na₂CO₃ solution and 0.25 mL of diluted (1:1) Folin–Ciocblteu reagent were added. The volume was brought to 25 mL with distilled water. One hour later the solutions were analyzed through a spectrophotometer (Unico 2804 UV, Suite E Dayton, NJ, USA), and the concentration of polyphenols was calculated according to the absorption of the reaction mixture at 730 nm. 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.).

2.6. Antioxidant Activity (AOA)

The antioxidant activity of Lobo leaves was assessed on 70% ethanolic extracts of dry leaf powder using a redox titration method [13]. The values were expressed in mg gallic acid equivalents (mg GAE g⁻¹ d.w.).

2.7. Ascorbic acid

The ascorbic acid content in leaves was determined by visual titration of plant extracts in 3% trichloroacetic acid with Tillman's reagent [14]. Three grams of fresh leaf homogenates were mixed with 5 mL of 3% trichloroacetic acid and quantitatively transferred to a measuring cylinder. The volume was brought to 60 mL using trichloroacetic acid, and the mixture was filtered through filter paper 15 min later. The concentration of ascorbic acid was determined from the amount of Tillman's reagent that went into titration of the sample.

2.8. Sugars

The monosaccharides were determined using the ferricyanide colorimetric method, based on the reaction of monosaccharides with potassium ferricyanide [15]. Total sugars were analogically determined after acidic hydrolysis of water extracts with 20% hydrochloric acid. Fructose was used as an external standard. The results were expressed in % per dry weight.

2.9. Total Dissolved Solids (TDS)

Portable conductivity meter TDS-3 (Korea) was used for the determination of the total dissolved solids.

2.10. Nitrates

Nitrates were assessed using ion-selective electrode with ionomer Expert-001 (Econix Inc., Moscow, Russia) according to [16].

2.11. Statistical Analysis

Data were processed by analysis of variance, and mean separations were performed through Duncan's multiple range test, with reference to the 0.05 probability level, using the SPSS software version 28 (Armonk, NY, USA).

3. Results and Discussion

Raphanus sativus lobo is easily infested by *Contarinia nasturtii* (Fig. 1). Single spraying of lobo plants by (1) sodium selenate solution, (2) Siliplant fertilizer as a source of ionic silicon and (3) garlic extracts during the period of active gall midge infestation resulted in 100% larvae mortality only one week later in cases of Siliplant application, either singly or in combination with sodium selenate and garlic extract supplementation (Table 1). The lack of a significant positive effect of sodium selenate supplementation may be connected both with the lower gall midge sensitivity to Se and/or the necessity



Fig. 1. *Raphanus sativus* var. *lobo* infested by *Contarinia nasturtii*
Рис. 1. Растение *Raphanus sativus* var. *lobo*, зараженное *Contarinia nasturtii*

for a longer period of exposure. Neither Se nor Si have been earlier used for plant protection against *C. nasturtii*. Contrary, garlic oil along with eucalyptus oil were shown to act as repellents for *C. nasturtii* [17]. Selenium protection against pests is connected with the toxic effect of the element, while silicon application along with the anti-feeding effect was shown to induce the emission of plant volatile attractants of natural predators and parasitoids [8, 18, 19].

Furthermore, it should be indicated that all three supplements (Se, Si and garlic extract) are known as powerful growth stimulators improving plant immunity, antioxidant defense and stimulating photosynthesis that positively affect seed productivity and quality. Thus, investigations on lettuce seed plants revealed the increase in seed productivity, proline and phenolic content increase due to foliar application of sodium selenate, sole or in combination with garlic extract [20]. In the present research mass of 1000 seeds varied from 13.5 to 25.2 g being the highest for plants treated with Siliplant separately or in combination with sodium selenate. Thus, the most promising method for lobo protection against *C. nasturtii* was Siliplant foliar supplementation.

Changes in biochemical characteristics of plants due to Se, Si and garlic extract application are directly connected with the improvement of plant adaptability and participation of the antioxidant defense system [8, 21].

Table 1. Seed productivity and *C. nasturtii* infestation levels under Se, Si and garlic extract administration under infestation.
Таблица 1. Семенная продуктивность и уровни инфицирования крестоцветной галлицей в условиях использования препаратов селена, кремния и экстракта чеснока

Parameter Параметр	Control Контроль	Siliplant Силиплант	Se	Se + Siliplant Se+ Силиплант	Garlic Чеснок
Seed productivity, g/plant Семенная продуктивность, г/растений	0.2*	22.3	6.1	20.3	10.4
Mass of 1000 seeds, g Масса 1000 семян, г	13.5	25.2	14.4	22.1	14.8
Presence of alive larvae Присутствие живых личинок	+++	-	+	-	-

'+++'- fully infested plants; '-' = 100% mortality of larvae, '+'- 50 % reduction of live larvae

Table 2. Dry matter, total dissolved solids (TDS), nitrate and ash content in lobo leaves.
Таблица 2. Содержание сухого вещества, водорастворимых соединений (TDS) и золы в листьях лобы

Parameter Параметр	Control Контроль	Siliplant Силиплант	Se	Se + Siliplant Se+Силиплант	Garlic Чеснок
Dry matter, % Сухое вещество, %	12.29 a	11.67 a	11.25 a	12.48 a	11.56 a
Water-soluble compounds TDS, mg/g Водорастворимые соединения, мг/г	57.1 b	72.8 a	70.4 a	56.3 b	58.0 b
Nitrates, mg/Kg d.w. Нитраты, мг/кг с.м.	2968 a	3112 a	3306 a	2326 b	2750 ab
Ash, % Зола, %	10.0 a	10.9 a	11.3 a	9.1a	9.1 a

Values in lines with similar letters do not differ statistically according to Duncan test at $p < 0.05$

Data presented in Table 2 indicate lack of significant Se, Si and garlic extract effect on the dry matter content of plants and ash content. Only Siliplant and Se application increased significantly the water-soluble compound content (TDS) exceeding that of the control plants by 1.23-1.27 times. The same tendency was registered for nitrate accumulation with the lowest values being typical to Se+Siliplant treated plants.

Antioxidant status of plants is directly connected with the protection against different stresses including insect pest infestation [21]. The results of the present work indicate lack of any differences in the total antioxidant activity between fully infested (control) plants and plants treated with Si, Se and garlic extract. Contrary, all treatments provided a significant increase in the polyphenol content with the highest positive effect of garlic extract confirming the known results of polyphenols importance in plant protection against herbivories [21]. It should be specially noted that the presented values of both total antioxidant activity (AOA) and total phenolics (TP) refer to the antioxidants soluble in 70% ethanol. Numerous investigations in optimization of polyphenol extraction conditions indicate significantly lower levels of TP in water extracts compared to ethanol utilization [22,23].

Among other biochemical parameters tested, a significant disaccharide content increase was recorded under all treatments, especially intensive in the case of garlic extract (Figure 5).

The detected phenomenon is directly connected with the active participation of carbohydrates in plant protection [24,25]. Infestation of plants by insect pests affect several

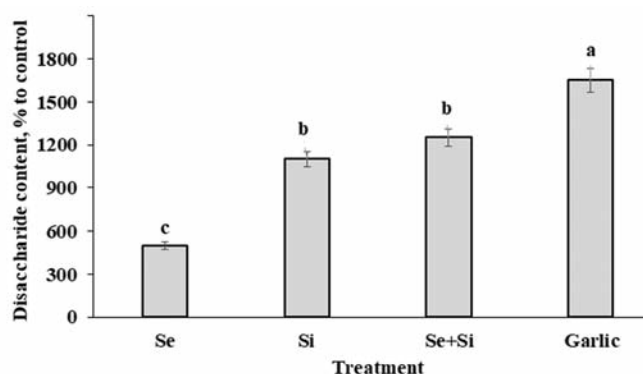


Fig. 2. Changes in disaccharide content in *Raphanus sativus* var. lobo treated with Siliplant, Se and garlic extract under *Contarinia nasturtii* infestation
Рис. 2. Изменения в содержании дисахаридов в инфицированных растениях лобо в результате обработок Силиплантом, селеном и экстрактом чеснока

secondary metabolites biosynthesis participating in plant resistance to the biotic stress [26,27]. In this respect, sucrose is known to participate in wounded tissues repair as an energy and carbon structure source and as a signal molecule for activation of the defense gene expression [28] and it is actively transported from injured sites to undamaged organs for growth recovery [29]. In this respect sucrose invertases and sucrose transporters are known to be highly affected by herbivory attack [30]. Thus, plant carbohydrate restoration due to Se, Si and garlic extract supply is reflected in a significant

Table 3. Effect of foliar application of Si (Siliplant), sodium selenate and garlic extract on biochemical characteristics of *Raphanus sativus* var. lobo under *Contarinia nasturtii* infestation.
Таблица 3. Влияние обработок лобы Силиплантом, селенатом натрия и экстрактом чеснока на биохимические характеристики растений в условиях инфицирования крестоцветной галлицей

Parameter Параметр	Control Контроль	Siliplant Силиплант	Se	Se + Siliplant Se+Силиплант	Garlic Чеснок
AOA*, mg GAE/g d.w. Антиоксидантная активность, мг-экв ГК/г с.м.	46.1a	44.1a	48.6a	46.1a	50.5a
TP**, mg GAE/g d.w. Полифенолы, мг-экв ГК/г с.м.	13.0b	15.0ab	16.0a	16.1a	17.1a
Se, µg/Kg d.w. Se, мкг/кг с.м.	146c	213b	1080a	1295a	150c
Monosaccharides, % Моносахара, %	17.0a	16.9a	12.6b	13.9b	16.5a
Disaccharides, % Дисахара, %	0.2d	1.0c	2.2b	2.5b	3.3a
Total sugar, % Общее содержание сахара, %	17.2ab	17.9a	14.8b	16.4ab	19.8a

* AOA— total antioxidant activity, **TP— polyphenols. Along each line, values with the same letters do not differ statistically according to Duncan test at $p < 0.05$

* AOA— общая антиоксидантная активность, **TP— полифенолы. Значения в рядах с одинаковыми индексами не различаются статистически согласно тесту Дункана при $p < 0.05$

increase in disaccharide content of the infested plants leaves with the highest effect typical for garlic extract application.

The lower efficiency of sodium selenate treatment compared to that of Siliplant and garlic extract, and the different intensities in biochemical changes (Table 2, Figure 4) indicate that Se protective effects require more time than those of Si and garlic extracts.

In conditions of the present experiment Se biofortification levels of lobo plants reached 7.4–8.9 indicating a tendency of the increase of Se assimilation due to Si supply which was in agreement with the results of the previous research on chervil [31]. Furthermore, significant increase in lobo leaf Se level in

plants treated with Siliplant without Se application confirms the existence of the Se-Si interaction phenomenon.

Conclusion

The results of the present investigation indicate high prospects of garlic extract, silicon and selenium supply for protection of *Raphanus sativus* var. lobo against *Contarinia nasturtii* attack. Further research is necessary to optimize the conditions of treatments and to confirm the efficiency of Se, Si, garlic extract supply for protection of other *Brassicaceae* representatives against infestation with *Contarinia nasturtii*.

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