

FUNGICIDAL AND GROWTH-STIMULATING EFFECT OF *RHEUM RHAPONTICUM* L ROOTS AND LEAVES PLANT EXTRACTS IN THE SOYBEAN SEEDS PRESOWING TREATMENT

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Summary: The aim of our research was the determination of the fungicidal and growth-stimulating effect of *Rheum rhaponticum* roots and leaves extracts in the soybean seeds presowing treatment. The methods of cultivation and research of *R. rhaponticum* plant materials presented in this work was carried out at the Institute of Genetics, Physiology and Plant Protection (2019). The subject of our study the bioactive substances of *R. rhaponticum* L. roots and leaves of manual collection were used. As an object of research were used phytopathogens of cereals, the genus *Neocosmospora* micromycetes, which are capable to cause damage to all vegetative organs and contaminating the grain with mycotoxins during the growing season and storage. It was found that the number of seedlings root nodules in the standard is 3,5 times less than in the control, while in variant V3 (0,5 % L + 1,5 % R) is 36,4 % more than in control. *R. rhaponticum* L. roots extract in natural background stimulated the germination of soybean seeds by 59,5 % in comparison with the control. It was determined that the biological effectiveness of composition V3 in the control of *Neocosmospora solani* (Mart.) L. Lombard & Crous phytopathogens of soybean seeds was 85,4 %, which exceeds the effectiveness of its constituent extracts individually and the standard in conditions of infected background. It was proved that the combination of root extracts and rhubarb leaves combines stimulating and fungicidal (biological effectiveness 85,4 %) properties for soybean seeds.

Key words: *Rheum rhaponticum*, *Neocosmospora solani* (Mart.) L. Lombard & Crous., stimulating effect, soybean seeds.

EVALUAREA PROPRIETĂȚILOR FUNGICIDE ȘI STIMULATOARE A EXTRACTELOR VEGETALE DIN RĂDĂCINILE ȘI FRUNZELE *RHEUM RHAPONTICUM* L DUPĂ TRATAREA SEMINȚELOR DE SOIA

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Rezumat: Scopul investigațiilor noastre au fost de a evalua proprietățile fungicide și stimulative a extractelor vegetale din rădăcinile și frunzele de *Rheum rhaponticum* la tratarea semințelor de soia înainte de încorporarea lor în sol. Experiențele de multiplicare, creștere, și studiere a materialului vegetal din *R. rhaponticum*, expuse în lucrarea dată, au fost efectuate pe câmpurile experimentale a Institutului de Genetică, Fiziologie și Protecție a Plantelor pe parcursul anilor 2012-2019. Obiectul investigațiilor a fost substanțele biologice active extrase din rădăcinile și frunzele de *R. rhaponticum*, colectate în mod manual. De asemenea, și organismele fitopatogene a culturilor cerealiere, micromicetele genului *Neocosmospora*, care provoacă atacul tuturor organelor vegetale și contaminarea boabelor cu microtoxine pe perioada de vegetație și depozitare. A fost demonstrat, că numărul tubercul rădăcină în varianta etalon era de 3,5 ori mai mic decât în varianta martor. Totodată, în varianta V3 (0,5% L+1,5% R) au fost cu 36,4% mai multe, decât în varianta

martor. Compoziția (V3) în condiții de câmp a influențat asupra stimulării germinării semințelor de soia cu 59,5% în comparație cu varianta martor. S-a constatat, că eficacitatea biologică a compoziției V3 în combaterea organismelor fitopatogene de *Neocosmospora solani* (Mart.) L. Lombard & Crous la semințele de soia a constituit 85,4%, cea ce e superior față de eficacitatea a fiecărui extract în parte și față de etalon, în condițiile de câmp. A fost demonstrat, că combinarea extractelor din rădăcinile și frunzele de *R. raponticum*, înclud în sine proprietăți stimulatoare și fungicide (eficacitatea biologică 85,4%) la tratarea semințelor de soia.

Cuvinte-cheie: *Rheum rhaponticum*, *Neocosmospora solani* (Mart.) L. Lombard & Crous, fungicide stimulatoare, semințe de soia.

INTRODUCTION

Phenolic compounds are valuable chemotaxonomic markers of the entire *Polygonaceae* family, and the synthesis of these various low molecular weight substances is a characteristic feature of their metabolism [1, 2]. These anthracene derivatives are able to induce systemic resistance by generating of reactive oxygen species, lignification of cell walls, the accumulation of PR-proteins, primarily chitinase and glucanase, as well as proteins, proteinase inhibitors, etc. This mechanism is activated mainly when protecting against biotrophic pathogens [3]. According to the classic of allelopathy the ability of some higher plants to resist fungal pathogens depends on the presence of emodin in their tissues. Emodin is a prophylactic toxin because it is constantly present in the plant and can directly affect the resistance of plants to diseases caused by microbial pathogens [4]. Activation of lignins in the cell walls and an increase in the activity and transcription of peroxidase, according to a scientist from the US Academy of Sciences, Bishop (2000) occurs when signs of infection appear during plants stress. The author indicates that peroxidase activity is widely associated with the polymerization of phenolic compounds, the precipitation of lignin (part of the rhubarb root extract), and the crosslinking of phenols with cell wall proteins. Lignin is part of the rhubarb root extract [5].

As a result of research (HPLC method) by A. Kostikova (2015) the features of flavonoid accumulation in the rhubarb leaf blades ethanol extracts, flavonol quercetin and its glycoside - rutin (6 %), were found [6]. Non-hormonal growth regulator - quercetin plays an important role in the regulation of dormancy and the induction of seed germination. It is part of the J3 inhibitor complex [7]. Being a component of a single plant antioxidant system, quercetin serves as a substrate of peroxidase and is oxidized by the enzyme in individual and joint oxidation reactions. However, the mechanism of this interaction is not well understood. At the same time, it was proved that the treatment of seeds with organic acids (in particular, oxalic), stably stimulates germination rates, due to their growth-promoting activity [8-10]. It is also known that the leaves of the genus *Rheum* plants accumulate about 3% of organic acids, of which oxalic acid – 1%. The aim of our research was the determination of the fungicidal and growth-stimulating effect of *Rheum rhaponticum* roots and leaves extracts in the soybean seeds presowing treatment.

MATERIALS AND METHODS

The methods of cultivation and research of *R. rhaponticum* plant materials presented in this work was carried out at the Institute of Genetics, Physiology and Plant Protection (2019). The subject of our study the bioactive substances of *R. raponticum* L. roots and

leaves of manual collection were used. As an object of research were used phytopathogens of cereals, the genus *Neocosmospora* micromycetes, which are capable to cause damage to all vegetative organs and contaminating the grain with mycotoxins during the growing season and storage. *Neocosmospora* grain damage causes its weakness, a sharp deterioration in the chemical-technological properties – a decrease in protein, gluten, and reduced flour quality. When using infected seeds for sowing, inhibition of seed germination, root growth and germination occurs. Field germination of seeds is reduced, surviving plants have an affected root system, vegetative and generative organs. Productivity is reduced to 50%.

Based on the presence of biologically active substances in the composition of the root and leaves of *R. rhaponticum* (emodin, quercetin), a scheme for their extraction was developed, consisting of collection, drying and grinding of plant material, mixing with a solvent (ethyl alcohol at a concentration of 70%), extraction into water bath, maceration (5-6 hours), evaporation and preparation of a soluble concentrate. Due to the presence of phenols and ethanol, the concentrate is well preserved.

The experiments were conducted with soybean in a greenhouse following the procedure developed by Hwang et al. (2006). The isolate of *Neocosmospora solani* (Mart.) L. Lombard & Crous was grown on potato dextrose agar plates and cultured for 5 days for inoculating wheat grain. One liter of grain was soaked overnight at room temperature (25° C) and rinsed three times with tap water. The autoclaved grain then was then inoculated with 5 pieces of 1×1 cm plug of the 5-day culture of *N. solani* per bag and cultured for 5 days at room temperature (25° C.). After the seeds were coated, they were left to air dry before planting. To prepare the soil, five hundred milliliters of the dried grains with inoculum were blended for 15 seconds three times and the powder was mixed with sterile sand at 1:1 (v/v) to dilute the inoculum. The sand mix was further used to prepare soil for. The seeds were planted in the pots with infested soil. There were 3 replicates of each treatment which were arranged in a complete randomized block design and placed at 25 to 30° C. in a greenhouse. After 30 days' emergence of each treatment was rated and compared.

The extracts stimulating properties were determined by seed germination, size and number of root nodules of seedlings in vegetation container with natural soil. The extracts fungicidal properties were determined by the level of disease intensity and the biological effectiveness of presowing treatment with rhubarb extracts in the vegetation container with infected soil. The seeds were treated with the indicated formulations for 15 min, in triplicate (20 plants in each repetition). The composition of the varieties of soybean («Nadejda»): control; Royal Flo standard; V 1 – 0,5% L; V 2 – 1% R; V 3 – 0,5% L + 1% R. Experience duration was 30 days. At the end of this period, the plants were removed from the vegetation container together with the root system and an assessment was carried out in accordance with surface damage of the root and stem (%). Based on the obtained data, the biological effectiveness of presowing treatment with rhubarb extracts and the optimal composition and concentration variant [26, 27] were determined.

The intensity, or degree, of plant damage (a qualitative indicator of the disease manifestation) was determined by the area of the plant organs affected surface or by the intensity of the disease manifestation symptoms.

Prevalence is the diseased plants number as a percentage of the total number examined on the site area. The prevalence (P, %) was calculated after counting sick and healthy plants in the sample according to the formula $P = \frac{100n}{N}$, where n – is the diseased plants number in the sample; N – is the total number of plants examined in the sample. The biological effectiveness of the protective measures was evaluated by comparing the plant damage on treated and control plants. The difference in prevalence of the control and treated plants (B, %) is determined by the $B = \frac{100 (P_c - P_e)}{P_c}$ formula, where P_c - is an indicator of the prevalence of the disease at a control plot; P_e - a similar indicator in the experimental plot [28]. Microsoft Office Excel software package was used to build graphic materials. Mathematical processing and reliability assessment of the obtained scientific data was carried out using the ABC Pascal platform.

RESULTS AND DISCUSSIONS

Investigation of the *R. rhaponticum* root and leaves extracts presowing treatment effect on the germination of soybean (Nadejda) was first carried out in laboratory conditions to determine the optimal concentration. The fungicidal preparation Royal FLO 42 SL was used as etalon, and in the control – seed treatment with water.

The treatment of seeds with a solution of rhubarb root and leaf extracts 0.5% L + 0.5% R stimulated the most stably all indicators of germination (Table 1). It was found that the effect of pre-sowing treatment with rhubarb root extract at a concentration of 1%, rhubarb leaf extract at a concentration of 0.5 %, and the composition of extracts (0,5 % L + 0,5 % R) on the germination and size of seedlings is stimulating for soybean seeds. The most stable stimulation of all germination indicators was the treatment of seeds with a composition of rhubarb root and leaves extracts 0,5 % L + 0,5 % R (Table 1).

A natural infectious background was used to determine the stimulating effect of presowing treatment of soybean with rhubarb extracts. An artificial infectious background was used to study the fungicidal properties of rhubarb extracts. The seeds were treated with the indicated formulations for 15 minutes; the seeds were sown in

Table 1. The effect of the *Rheum rhaponticum* roots (R) and leaves (L) extracts on the germination rate of seeds and the size of the soybean seedlings in the laboratory

Variant	Germination energy, %	Germination, %	Seedling root sizes, sm	Seedling stem sizes, sm
Control	38,3	48,3	1,7	0,4
Etalon	55,0	73,3	2,1	1,3
V1 – 0,5 % L	63,3	80,0	1,6	1,2
V2 – 1 % L	43,3	68,3	1,1	1,2
V3 – 1 % R	61,7	83,3	1,8	2,0
V4 – 3 % R	43,3	78,3	1,8	1,9
V5 – 0,5 % L + 0,5 % R	56.7	90	1,9	2,1
DEM _{0,05}	12,4	13,6	0,6	0,3

vegetation containers in 3 repetitions (20 plants in each repetition). Germination rates were determined. For evaluation of the intensity of damage to plants by root rot, we used a 5-point scale (CIMMYT) [22].

The stimulating properties of the extracts were determined by seed germination, size and number of root nodules of seedlings in natural soil. Seed treatment with a composition of rhubarb leaf and root extracts (V3) increased soybean germination by 46,6 %, and the number of root nodules by 36,4 % compared with the control. Moreover, the number of



Figure 1. General view of options planted on an infectious background after treatment of soybean seeds with rhubarb extracts



Figure 2. Assessment of the intensity of damage to soybean roots by rot on a 5-point scale (1 = 1-9%, 2 = 10-29%, 3 = 30-69%, 4 = 70-89%, 5 = 90-99%)

root nodules in the standard is 71,4 % less than in the control. Presowing treatment with rhubarb root extract (V2) increased soybean germination by 25 %, compared to control. It was found that rhubarb leaf extract at a concentration of 0,5 % increased soybean germination by 48,3 % (Table 2).

The fungicidal properties of the extracts were determined by the level of disease intensity and the number of seeds not germinated in the infected soil. As a result of experiments, it was found that the combination of rhubarb root and leaves extracts has the

Table 2. The effect of presowing treatment of soybean seeds (Nadejda) with *R. rhaponticum* extracts from the root (R) and leaves (L) on the natural seed germination, size and number of root nodules of seedlings in natural background

Variant	Natural background		
	seed germination, %	Seedling stem sizes, sm	number of seedlings root nodules, nod./plant
Control	31,7	41,5	0,7
Etalon	45,0	41,6	0,2
V1- 0,5 % L	80,0	42,3	0,7
V2- 1,5 % R	56,7	42,7	0,8
V3- 0,5 %L+1,5 % R	78,3	45,3	1,1
DEM _{0,05}	15,9	2,2	0,2

maximum fungicidal properties in the suppression of soybean phytopathogens *N. solani*. The biological effectiveness of the composition (85,4%) is higher than the effectiveness of its constituent extracts separately. The biological effectiveness of presowing treatment with rhubarb root extract (V2) was 70,8% (Figure 3).

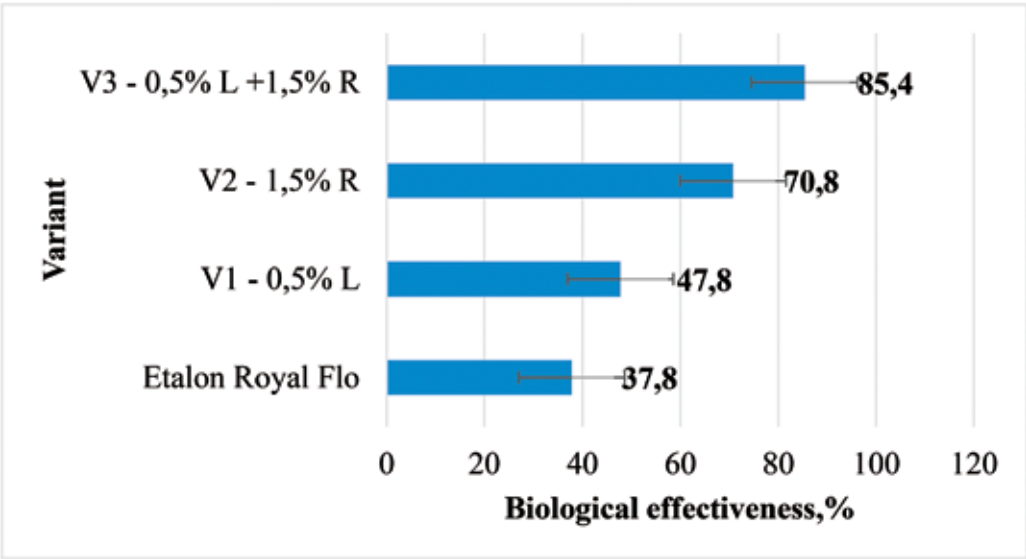


Figure 3. Biological effectiveness of presowing treatment of soybean seeds (Nadejda) with *R. rhaponticum* root (R) and leaves (L) extracts from an artificial infectious (*N. solani*) background (DEM_{0,05} - 17,6)

CONCLUSIONS

1. It was found that the number of seedlings root nodules in the standard is 3,5 times less than in the control, while in variant V3 – 0,5 % L + 1,5 % R is 36,4 % more than in control. Composition (V3) in natural background stimulated the germination of soybean seeds by 59,5 % in comparison with the control.

2. It was determined that the biological effectiveness of composition V3 (0,5 % L + 1,5 % R) in the control *N. solani* phytopathogens of soybean seeds was 85,4 %, which exceeds the effectiveness of its constituent extracts individually and the standard in conditions of infected background.

3. It was proved that the combination of root extracts and rhubarb leaves (0,5% L + 1,5 % R) combines stimulating and fungicidal (biological effectiveness 85,4 %) properties for soybean seeds.

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