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INFLUENCE OF SURFACE-ACTIVE RHAMNOLIPID BIOCOMPLEX AND ETHYLTHIOSULFANILATE ON GROWTH AND BIOCHEMICAL VALUES OF PLANTS IN THE OIL CONTAMINATED SOIL

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The aim of the work was to study the influence of rhamnolipid biocomplex and ethylthiosulfanilate on field pea and sorghum plants when growing in petroleum contaminated soil. Plant seeds were treated with solutions of rhamnolipid biocomplex or ethylthiosulfanilate (0.01 g/l) before planting and grown in containers with soil artificially contaminated with petroleum (5, 8 and 10%). Effect of rhamnolipid biocomplex and ethylthiosulfanilate was determined by the determination of growth (weight, length plants) and biochemical parameters (content of photosynthetic pigments, hydrogen peroxide, malondialdehyde).

The stimulating effect of rhamnolipid biocomplex and ethylthiosulfanilate on growth parameters of field pea was shown: the shoot mass has significantly increased on 39%, root mass — on 26% if compared with the control. For sorghum somewhat smaller increase of growth parameters was observed. Under the influence of rhamnolipid biocomplex and ethylthiosulfanilate the content of photosynthetic pigments in field pea and sorghum has also increased. It was determined that the action of biocomplex and ethylthiosulfanilate promoted the decrease of indicators of plant oxidative reactions if compared with control: the content of hydrogen peroxide — in average on 15% and 16%, malondialdehyde — on 13.5% and 16% respectively.

The results of the study testify to the effectiveness of rhamnolipid biocomplex and ethylthiosulfanilate as growth stimulators for field pea and sorghum, as well as improvement of adaptive capability of plants to unfavorable conditions. It creates the prospects of their application as effective and ecologically safe substances for the intensification of contaminated soil phytoremediation.

Key words: rhamnolipid biocomplex, ethylthiosulfanilate, field pea, sorghum, petroleum.

Recently, new promising, economically viable and environmentally safe biotechnological methods of remediation of contaminated areas, are becoming particularly important. Continuously growing influence is attracted to phytoremediation technologies with application of stable plants and growth stimulators [1]. Growth regulators can have stimulating effect on plant growth and influence their resistance to adverse conditions, in particular soil contamination of various nature [2, 3]. According to the literature, the use of preparations Emistim C and Agrostimulin in pre-sowing treatment of seeds helped to accelerate the development of plants and improve their adaptation to stressful environmental conditions [4].

In our opinion, biogenic surfactants (biosurfactants) can be promising agents for the improvement of plant resistance to adverse conditions due to their high efficiency and unique physical, chemical and biological properties. Biosurfactants have several advantages over synthetic surfactants, since they are biodegradable and low toxic, which determines their use in environmentally safe technologies [5]. The ability of biosurfactants to influence the permeability of cell membranes, activity of enzymes, and other biologically active substances promotes plant growth [6].

It is known that biosurfactants can be used for remediation of contaminated areas, including soil, contaminated with petroleum and petroleum products [7]. Not only petroleum but also the pathogenic fungi, present in the contaminated soils, inhibit plant growth and the use of biocides is also appropriate to protect plants in these conditions [8]. Biocide ethylthiosulfanilate (ETS) — a synthetic analogue of phytoncide allicin of garlic or onion — belongs to disulfur-containing organic compounds and has a broad spectrum of antimicrobial activity [9]. At low concentrations ETS may act as plant growth regulator [10]. However, its effect on the growth of plants in petroleum contaminated soils was not investigated.

An important role in the adaptation of plants to unfavorable conditions belongs to photosynthesis pigments and antioxidant biochemical reactions [11]. They participate in neutralizing reactive oxygen species (ROS), which excessively accumulate in plant cells under the influence of stressors and initiate the processes of oxidative degradation of membrane structures with formation of hydrogen peroxide and malondialdehyde (MDA) — a product of lipid peroxidation (LPO) which affects proteins and DNA. However, ROS and LPO products may play the role of signaling molecules involved in the activation of protective systems in cells.

In turn, hydrogen peroxide is considered as a messenger in the transmission of cellular signals, because it is a small, short-lived, freely diffusing molecule that is generated as a result of enzymatic reactions in response to the stress signal and affects thiol groups of proteins [12]. Hydrogen peroxide is accumulated in plant cells due to photorespiration and redox processes and in excess amounts has a toxic effect on cells. In this regard, the destruction of hydrogen peroxide is important for preventing toxic effect on cells and for the formation of water and oxygen, vital for plants.

Based on the above, the aim of the work was to study the influence biosurfactants of rhamnolipid nature and biocide ethylthiosulfanilate on growth (morphometric) and biochemical parameters of plants, which characterize the adaptive processes when growing on contaminated soils.

Materials and Methods

Seeds of field pea (*Pisum arvense* L.) and sorghum sudangrass (*Sorghum bicolor subsp. drummondii*), and biological preparations — ethylthiosulfanilate (a synthetic analogue of garlic phytoncides from the Department of Technology of Biologically Active Compounds, Pharmacy and Biotechnology of the Lviv Polytechnic National University) [9, 10], and rhamnolipid biocomplex (RBC), which is the product of microbial synthesis of the strain *Pseudomonas* sp. PS-17 containing rhamnolipid surfactants and polysaccharide (4:1) obtained in the Department of Physical Chemistry of Fossil Fuels of the Litvinenko Institute of Physical-Organic and Coal Chemistry of the National Academy of Sciences of Ukraine) were used in the work [6, 13]. The work was carried out with artificially spiked soils with different petroleum content (5%, 8% and 10%) as absolute control with clean garden soil.

Pre-sowing seed treatment was carried by the conventional method [14]: the seeds were soaked for 3 hours in solutions (0.01 g/l) of RBC and ETS, the control variant was soaked in water, then planted in containers, each containing 300 g of soil.

Plants were grown in the laboratory conditions at 18–20 °C under fluorescent light (fluorescent lamp spectrum maximum — 0.50–0.66 nm) during 21 days, photoperiod was 12 hours of light and 12 of darkness. After that morphometric (weight of shoot and root, length of the plants) and biochemical parameters (content of photosynthetic pigments, hydrogen peroxide, malondialdehyde) were determined.

Plant photosynthesis pigments were determined by spectrophotometric method, extraction of pigments was carried out with acetone. Absorbance was determined at 662 nm (for chlorophyll *a*), 644 nm (for chlorophyll *b*) and 440.5 nm (for carotenoids) using the spectrophotometer Shimadzu UVmini-1240 (Shimadzu Corporation, Japan), their content was calculated using Holm-Wettstein formulas [15] in mg/g of wet matter weight.

Malondialdehyde content in plants during lipid peroxidation (LPO) was determined by its interaction with 2-thiobarbituric acid, which resulted in formation of colored product with maximum absorption at 532 and 600 nm [16]. MDA content was expressed in mol/g of wet matter weight.

The hydrogen peroxide content was measured by spectrophotometric method in plant homogenate after centrifugation [17]. 1 ml of the supernatant was supplemented with 3 ml of 0.1% $Ti(SO_4)_2$, the color intensity was assessed at 410 nm, H_2O_2 content was expressed as mM/g of wet matter weight. The experiments were performed in triplicates. Statist and Microsoft Exel software were used for for statistical data processing.

Results and Discussion

To study the influence of ETS and RBC on plants the artificially spiked soil with different petroleum content (5%, 8% and 10%) was used. Such model pollution was selected according to a range of petroleum concentrations occurring in the soil at sites subject to reclamation (including the territories of petroleum companies in Western Ukraine).

The optimum concentration of RBC and ETS solutions by their effect on plants were identified in previous studies [6, 10].

In the first stage the influence of ETS and RBC on morphometric parameters of the growth of plants on soil with different petroleum concentrations were studied. When comparing growth parameters of the studied plants the stimulating effect of the action of both RBC and ETS was observed, but the best results were shown for field pea: the mass of plants which is an integral factor of their growth and development, increased for shoot on 39% and for root — on 26% (Fig. 1, *A*).

Plant dimensions also increased: shoot length — on 35% in average, the root length — on 48% (Fig. 1, *B*).

In experimental variants with sorghum the increase of morphometric parameters under the influence of ETS and RBC was observed (Fig. 2).

As it is seen from the results the stimulating effect of ETS and RBC for sorghum was rather lower than for field pea.

The influence of rhamnolipid surfactants on plants can be explained primarily by their effect on the permeability of cell membranes, which helps to improve the absorption of water and nutrients from the soil. Biosurfactants may also stimulate the growth of plant cells by stretching, which was shown earlier in the biotests on wheat coleoptile cuts [6]. It is known that the effect of increase in cell stretching and weight is associated with better water absorption, activation of membrane enzymes, including H+ATPase and acid hydrolases [18]. Thiosulfanilates are highly reactive compounds interacting with nucleophiles, electrophiles, radicals.

Nucleophilic substitution reactions occur with an opening of −S-S-bond due to redistribution of electron density in thiosulfogroup that determines the direction of nucleophile attacks [9]. Presumably, ETS can contribute to the metabolism of RNA and DNA, which contain amino groups, as well as metabolism of proteins and amino acids with disulfide and sulfide fragments, which are the components of plant cells. This can cause accelerated cell division affecting enzymatic processes. The ETS influence on plants is obviously closely related with its protective action, participation in redox processes, contributing to violations of respiratory system of pathogenic microorganisms. At that thiosulfanilates have low toxicity (LD_{50} = 2000 mg/kg). In our opinion, in the case of ETS application for pre-sowing treatment of seeds it doesn't accumulate in the soil. It was shown that at low concentrations ETS can improve seed germination, weight of seedlings, reduce the number of diseased plants [10].

Under the influence of RBC and ETS the content of photosynthetic pigments increased in both plants (Fig. 3, 4), which are important indicators of plant metabolism both in normal and in stress conditions [19, 20].

A *B*

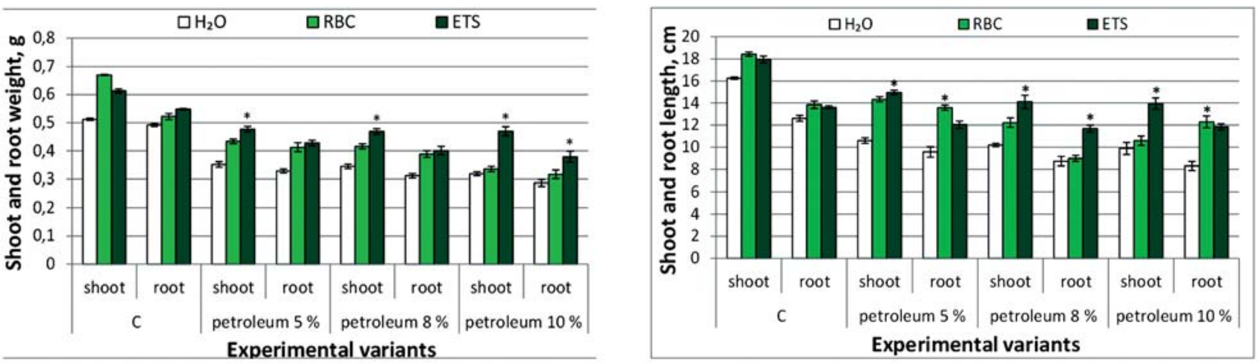

Fig. 1. **Morphometric parameters of field pea under the influence of rhamnolipid biocomplex and ethylthiosulfanilate when growing on petroleum contaminated soil:**
A — weight of shoot and root; *B* — length of shoot and root; *C* — absolute control — garden soil; * — $P \geq 0.05$ if compared with control

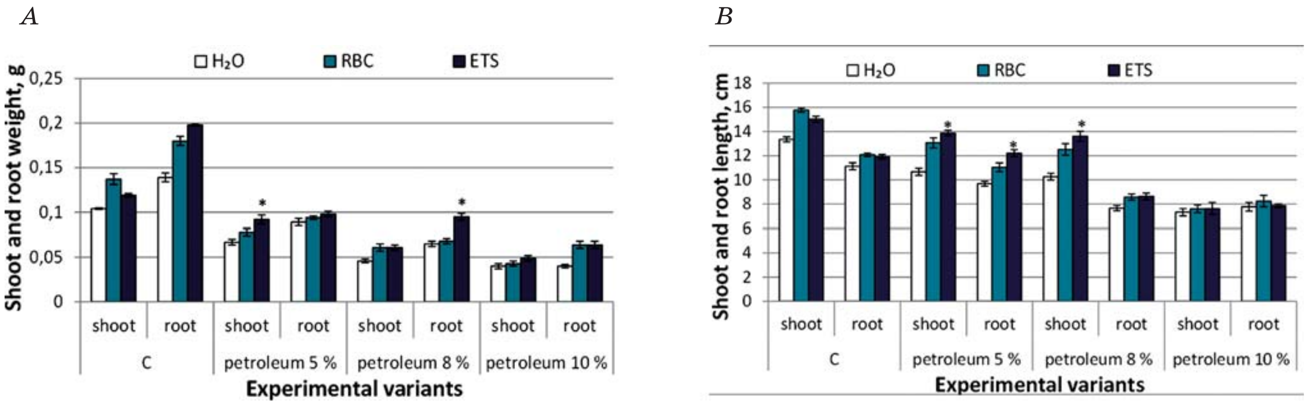

Fig. 2. **Morphometric parameters of sorghum under the influence of rhamnolipid biocomplex and ethylthiosulfanilate when growing on petroleum contaminated soil:**
A — weight of shoot and root; *B* — length of shoot and root; *C* — absolute control — garden soil; * — $P \geq 0.05$ if compared with control

Thus, increase in total chlorophyll *a+b* content in field pea under the influence of RBC and ETS when growing in petroleum contaminated soil was established: 5% contamination — on 9% and 5% respectively; 8% contamination — 34% and 27%; 10% contamination — 14% and 7% if compared to control (Fig. 3), which may indicate an increase of shade-tolerance of plants. This is confirmed by the literature data on the relationship between the total chlorophyll content in plants and their tolerance to low insolation [19] and is of great importance for plant adaptation, particularly in the Western region of Ukraine, which is characterized by a significant amount of dark, overcast days.

For sorghum the increase of total chlorophyll *a+b* content under the influence of RBC and ETS was observed: 5% contamination — on 20% and 10% respectively; 8% contamination — on 21% and 7%; 10% contamination — 22% and 16% if compared to control (Fig. 4). The established increase of chlorophyll *a+b* content in both plants may indicate the photosynthesis stimulation, which is probably connected with improvement of germination conditions caused by the action of RBC and ETS.

To evaluate the intensity of redox processes that characterize the negative impact of environmental factors on plants, the content of hydrogen peroxide and malondialdehyde were determined (Table 1, 2).

Thus, it was found that the content of these indicators in field pea (in the case of seed treatment with RBC and ETS solutions) has decreased: hydrogen peroxide — on 23% and 22% respectively, malondialdehyde — on 16% and 18% respectively compared to control (Table 2).

The reduction of the studied parameters was observed in sorghum plants after the treatment of seeds with RBC and ETS solutions: hydrogen peroxide content — on 8% and 10,6%, respectively, malondialdehyde —

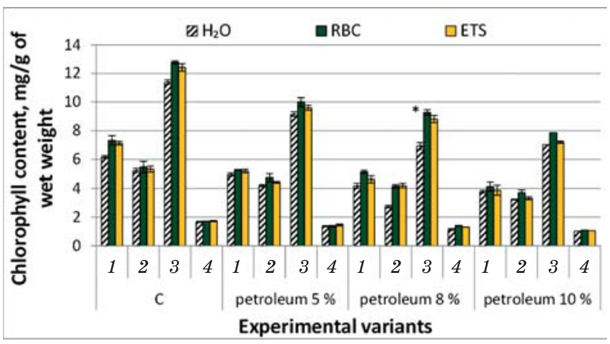

Fig. 3. **Content of photosynthetic pigments in field pea under the influence of rhamnolipid biocomplex and ethylthiosulfanilate when growing on petroleum contaminated soil:**
1 — chlorophyll *a*; *2* — chlorophyll *b*; *3* — total chlorophyll content *a+b*; *4* — carotenoids

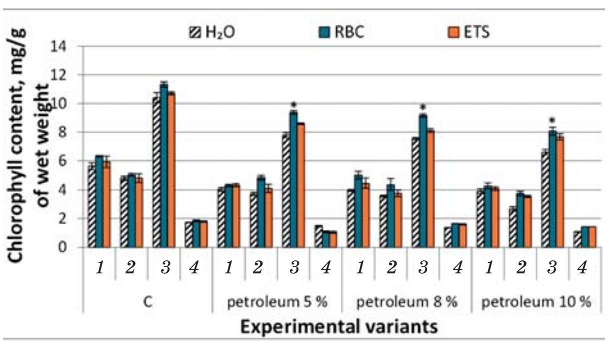

Fig. 4. **Content of photosynthetic pigments in sorghum under the influence of rhamnolipid biocomplex and ethylthiosulfanilate when growing on petroleum contaminated soil:**
1 — chlorophyll *a*; *2* — chlorophyll *b*; *3* — total chlorophyll content *a+b*; *4* — carotenoids

Table 1. **Content of MDA and hydrogen peroxide in field pea plants grown on petroleum contaminated soils**

Experimental variants	**H_2O_2, mcM/g of wet weight**	**Malondialdehyde, mcM/g of wet weight**
Control		
Control	1.92 ± 0.06	0.694 ± 0.032
Petroleum 5%		
Control	2.50 ± 0.05	0.909 ± 0.029
Rhamnolipid biocomplex	2.37 ± 0.07	0.753 ± 0.044
Ethylthiosulfanilate	2.30 ± 0.01	0.742 ± 0.024
Petroleum 8%		
Control	3.19 ± 0.08	1.011 ± 0.030
Rhamnolipid biocomplex	2.31 ± 0.10	0.882 ± 0.011
Ethylthiosulfanilate	2.48 ± 0.03	0.844 ± 0.010
Petroleum 10%		
Control	3.54 ± 0.07	1.140 ± 0.037
Rhamnolipid biocomplex	2.78 ± 0.11	0.985 ± 0.021
Ethylthiosulfanilate	2.72 ± 0.13	0.993 ± 0.013

Note: $n = 30$; all values are significantly different from control $P \geq 0.05$.

Table 2. **Content of MDA and hydrogen peroxide in sorghum plants grown on petroleum contaminated soils**

Experimental variants	**H_2O_2, mcM/g of wet weight**	**Malondialdehyde, mcM/g of wet weight**
Control		
Control	2.04 ± 0.05	0.613 ± 0.032
Petroleum 5%		
Control	2.06 ± 0.09	0.839 ± 0.056
Rhamnolipid biocomplex	1.91 ± 0.11	0.753 ± 0.047
Ethylthiosulfanilate	1.86 ± 0.06	0.704 ± 0.035
Petroleum 8%		
Control	2.58 ± 0.15	1.086 ± 0.075
Rhamnolipid biocomplex	2.39 ± 0.06	0.914 ± 0.023
Ethylthiosulfanilate	2.34 ± 0.05	0.903 ± 0.032
Petroleum 10%		
Control	3.03 ± 0.20	1.168 ± 0.031
Rhamnolipid biocomplex	2.81 ± 0.03	1.120 ± 0.025
Ethylthiosulfanilate	2.70 ± 0.09	1.103 ± 0.038

Note: $n = 30$; all values are significantly different from control $P \geq 0.05$.

on 11.3% and 15%, respectively, if compared to control (Table. 2).

According to the results, when cultivating the plants in contaminated soil oxidative reactions were activated (increase of MDA and H_2O_2), but after the seed treatment with RBC and ETS solutions, these parameters were significantly reduced, which could point to reduction of the impact of pollution. Since the intensity of oxidative reactions in field pea has decreased under the influence of RBC and ETS more than in sorghum, it can be assumed that field pea has better adaptive capacity for soil contamination.

Thus, the stimulating influence of RBC and ETS on the growth of field pea and sorghum on petroleum contaminated soils was determined: in field pea the shoot weight increased on 39%, root weight — on 26%, shoot length — on 35% and root length — on 48% if compared with control. The content of photosynthetic pigments increased in field pea and sorghum under the influence of RBC and ETS: chlorophyll *a* — on 12.8% and 6.6% respectively, chlorophyll *b* — on 28.1% and 17.8%, chlorophyll *a+b* — on 19.5% and 11%, carotenoids — 8.3% and 6.4%. The decrease of lipid peroxidation parameters in field pea and sorghum as a result of RBC and ETS application was established: the of hydrogen peroxide content — on 15% and 16% respectively, malondialdehyde content — 13.5% and 16%, indicating increased resistance of plants to unfavorable conditions of contaminated soil.

The obtained results indicate the prospects of application of biosurfactants and thiosulfanilate biocides as efficient and environmentally friendly substances for stimulation of phytoremediation of technological contaminated areas.

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ВПЛИВ ПОВЕРХНЕВО-АКТИВНОГО РАМНОЛІПІДНОГО БІОКОМПЛЕКСУ ТА ЕТИЛТІОСУЛЬФАНІЛАТУ НА РОСТОВІ ТА БІОХІМІЧНІ ПОКАЗНИКИ РОСЛИН НА ҐРУНТІ, ЗАБРУДНЕНОМУ НАФТОЮ

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Метою роботи було дослідження впливу рамноліпідного біокомплексу та етилтіосульфанілату на рослини гороху польового та сорго за вирощування на забрудненому нафтою ґрунті. Насіння рослин перед посівом обробляли розчинами рамноліпідного біокомплексу або етилтіосульфанілату (0,01 г/л) й вирощували в ємностях із ґрунтом, штучно забрудненим нафтою (5, 8 і 10%). Вплив рамноліпідного біокомплексу та етилтіосульфанілату визначали за ростовими (маса, довжина рослин) і біохімічними (вміст пігментів фотосинтезу, пероксиду водню, малонового діальдегіду) параметрами.

Показано стимулювальний вплив рамноліпідного біокомплексу та етилтіосульфанілату на ростові показники гороху польового: маса пагона достовірно збільшилася на 39%, кореня — на 26% порівняно з контролем. Для сорго спостерігався дещо менший приріст показників росту. За впливу рамноліпідного біокомплексу та етилтіосульфанілату в гороху і сорго також збільшувався вміст пігментів фотосинтезу. Встановлено, що за дії рамноліпідного біокомплексу та етилтіосульфанілату показники оксидативних реакцій рослин щодо контролю знижувалися: вміст пероксиду водню — у середньому на 15% і 16%, малонового діальдегіду — на 13,5% і 16% відповідно.

Результати дослідження свідчать, що рамноліпідний біокомплекс та етилтіосульфанілат є ефективними стимуляторами росту гороху польового і сорго, а також сприяють підвищенню адаптаційної здатності рослин до несприятливих умов. Це створює перспективи використання їх як ефективних та екологічно безпечних речовин для інтенсифікації процесу фіторемедіації забруднених ґрунтів.

Ключові слова: рамноліпідний біокомплекс, етилтіосульфанілат, горох польовий, сорго, нафта.

ВЛИЯНИЕ ПОВЕРХНОСТНО-АКТИВНОГО РАМНОЛИПИДНОГО БИОКОМПЛЕКСА И ЭТИЛТИОСУЛЬФАНИЛАТА НА РОСТОВЫЕ И БИОХИМИЧЕСКИЕ ПОКАЗАТЕЛИ РАСТЕНИЙ НА ПОЧВЕ, ЗАГРЯЗНЕННОЙ НЕФТЬЮ

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Целью работы было исследование влияния рамнолипидного биокомплекса и этилтиосульфанилата на растения гороха полевого и сорго при выращивании на загрязненной нефтью почве. Семена растений перед посевом обрабатывали растворами рамнолипидного биокомплекса или этилтиосульфанилата (0,01 г/л) и выращивали в емкостях с грунтом, искусственно загрязненным нефтью (5, 8 и 10%). Влияние рамнолипидного биокомплекса и этилтиосульфанилата определяли по ростовым (масса, длина растений) и биохимическим (содержание пигментов фотосинтеза, пероксида водорода, малонового диальдегида) параметрам.

Показано стимулирующее влияние рамнолипидного биокомплекса и этилтиосульфанилата на ростовые показатели гороха полевого: масса побега достоверно увеличилась на 39%, корня — на 26% по сравнению с контролем. Для сорго наблюдался несколько меньший прирост показателей роста. Под влиянием рамнолипидного биокомплекса и этилтиосульфанилата у гороха и сорго также увеличивалось содержание пигментов фотосинтеза. Установлено, что при действии рамнолипидного биокомплекса и этилтиосульфанилата показатели оксидативных реакций растений по сравнению с контролем снижались: содержание пероксида водорода — в среднем на 15% и 16%, малонового диальдегида — на 13,5% и 16% соответственно.

Результаты исследования свидетельствуют, что рамнолипидный биокомплекс и этилтиосульфанилат оказывают стимулирующее действие на растения гороха полевого и сорго, а также способствуют повышению адаптационной способности растений к неблагоприятным условиям. Это создает перспективы использования их как эффективных и экологически безопасных веществ для интенсификации процессов фиторемедиации загрязненных почв.

Ключевые слова: рамнолипидный биокомплекс, этилтиосульфанилат, горох полевой, сорго, нефть.