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Sotnikova Yu.M.[1], Grigoriadi A.S.[2], Fedyaev V.V.[3], Novoselova E.I.[4], Yamaleeva A.A.[5], Gabidullina G.F.[6], Farkhutdinov R.G.[7] *

[1, 2, 3, 4, 5, 6, 7] Bashkir State University, Ufa, Russia

* Correspodning author (frg2[at]mail.ru)

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APPLICATION OF MICROBIOLOGICAL PREPARATIONS AND LUCERN PLANTS FOR PHYTOREMEDIATING ACTIVITIES ON OIL CONTAMINATED SOILS

Research article

Abstract

Currently, phytoremediation is one of the promising methods for cleaning and restoring oil-contaminated lands. The use of microbiological preparations-oil destructors has also shown its effectiveness in remediation processes. However, the joint use of plant objects and microbiological preparations has not been studied enough. Thus, the search for microbial-plant associations consisting of a plant-phytoremediant, growth-stimulating microorganisms and microbes-destructors of oil hydrocarbons, the study of the mechanisms of their interaction and the effect of biological preparations on growth and physiological-biochemical processes in plants-phytoremediants, is one of the urgent tasks in the development technologies for reclamation of lands contaminated with oil and oil products. In this regard, we assessed the physiological and biochemical parameters of alfalfa plants against the background of the use of microbiological preparations under conditions of oil pollution of soils.

Keywords: alfalfa, phytoremidia, oil pollution, microbiological preparations.

Сотникова Ю.М.[1], Григориади А.С.[2], Федяев В.В.[3], Новоселова Е.И.[4], Ямалеева А.А.[5], Габидуллина Г.Ф.[6], Фархутдинов Р.Г.[7] *

[1, 2, 3, 4, 5, 6, 7] Башкирский государственный университет, Уфа, Россия

* Корреспондирующий автор (frg2[at]mail.ru)

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ПРИМЕНЕНИЕ МИКРОБИОЛОГИЧЕСКИХ ПРЕПАРАТОВ И РАСТЕНИЙ ЛЮЦЕРНЫ ДЛЯ ФИТОРЕМЕДИАЦИОННЫХ МЕРОПРИЯТИЙ НА НЕФТЕЗАГРЯЗНЕННЫХ ПОЧВАХ

Научная статья

Аннотация

В настоящее время фиторемедиация является одним из перспективных методов очистки и восстановления нефтезагрязненных земель. Применение микробиологических препаратов-нефтедеструкторов также показало свою эффективность в процессах ремедиации. Однако совместное использование растительных объектов и микробиологических препаратов изучено недостаточно. Таким образом, поиск микробно-растительных ассоциаций, состоящих из растения-фиторемедианта, ростостимулирующих микроорганизмов и микробов-деструкторов углеводородов нефти, изучение механизмов их взаимодействия и влияния биопрепаратов на ростовые и физиолого-биохимические процессы у растений-фиторемедиантов, является одной из актуальных задач при разработке технологий по рекультивации земель, загрязненных нефтью и нефтепродуктами. В этой связи нами был проведена оценка физиолого-биохимических параметров растений люцерны посевной на фоне применения микробиологических препаратов в условиях нефтяного загрязнения почв.

Ключевые слова: люцерна посевная, фиторемидиация, нефтяное загрязнение, микробиологические препараты.

1. Introduction

The natural restoration of oil-contaminated lands can take decades; therefore, it is important to find effective, inexpensive and safe methods for cleaning up and restoring contaminated lands. One of such methods in Russia and abroad is bioremediation, which includes the use of microorganisms that decompose oil hydrocarbons and plants capable of absorbing and transforming toxicants and activating the activity of soil microorganisms [1], [2], [3]. One of the most important indicators of the phytoremedial ability of a plant is its ability to accumulate the biomass of the aboveground part, which is capable of accumulating soil pollutants, and which can be easily utilized [4], [5].

2. Objects and methods of research

Plants of alfalfa (*Medicago sativa* L., cultivar Nadezhda) were chosen as objects of study. Plants were grown in vegetative vessels with a volume of 0,5 l, at a 14-hour light period, an illumination intensity of 24 klx, an air temperature of 22–25 °C, and 60% moisture from the total moisture capacity of the soil.

Biological preparations produced by CJSC NPP Biomedkhim (Ufa) were used in the work: Lenoil (*Pseudomonas turukhanskensis* IB 1,1 (1×10^8 CFU/g), Elena (*Pseudomonas aureofaciens* IB 51 ($2\text{-}3 \times 10^9$ CFU/ml), and "Agrobiologist" (*Pseudomonas protegens* DA1.2 (45×10^9 CFU/ml) - development of the Ufa Institute of Biochemistry, Ural Federal Research Center of the Russian Academy of Sciences (Ufa). In the work, the doses of the preparations recommended by the manufacturers were used.

The total protein content was determined according to Bradford [6]. The activity of guaiacol peroxidase was calculated according to the method of A.I. Ermakova (1987) [7]. Catalase activity was assessed by the method of M.A. Korolyuk (1988) [8]. To determine the activity of superoxide dismutase (SOD), the method of Chevari (1985) [9] was used. The concentration of adrenochrome in the samples was calculated according to F.V. Minibaeva and colleagues (2003) [10]. Determination of the content of hydrogen peroxide in plant material was carried out according to the method of Bindschedler (2001) [11] and Chaouch (2010) [12].

Biological and analytical repeatability in experiments is 3–5 times. The arithmetic mean and its error, standard deviation was calculated. To identify significant differences between the compared indicators, Student's t-test or analysis of variance was used. Differences were considered significant at a significance level of $p < 0,05$.

3. Results and discussion

Our studies have shown that the Lenoil biopreparation, when applied to oil-contaminated soil, stimulates growth processes in alfalfa and rye plants [13], [14]. The stimulation process is primarily associated with a decrease in the toxic effect of petroleum products due to their degradation by microorganisms of the Lenoil biological product [15]. The stimulating effect of the drug itself on plants growing on soils that do not contain petroleum products is not so small [15]. Previously, we found that the use of stimulating biological preparations on oil-contaminated soils in legumes of the preparation "Elena" led to the activation of growth processes [13], [14]. Therefore, it was a completely logical scheme for the use of biological products on oil-contaminated soils, when at first the Lenoil preparation would be introduced into the soil for the biodegradation of petroleum products, and then after a certain time the Elena biological preparation would be added to the soil for alfalfa. However, as our studies have shown, such a scheme was not entirely successful, in alfalfa - "oil + Lenoil + Elena", we found inhibition of growth processes [13], [14]. This could be due to a certain antagonism between the microorganisms of the applied biological preparations or from the natural microflora of the gray forest soil with the microorganisms of the biological preparations [16]. In this regard, we decided to change the method of processing plants - pre-sowing treatment of seeds and the introduction of the biopreparation "Lenoil" into the soil + spraying the plants with the microbiological preparation "Elena" or "Agrobiologist".

Estimating and comparing the length of shoots of alfalfa plants grown on oil-contaminated soils and plants of the control variant, we found that when plants grew in soil treated with the Lenoil biological product and which were sprayed with the Elena biological product, the shoot length was the same as in the control variant (Table 1). And along the length of the roots, this variant was 24% longer than the control. In terms of shoot mass, the Lenoil+Elena variant was 37% heavier than the control. In terms of root mass, the values of the control variant were almost 20% higher than those of the Lenoil+Elena variant.

Table 1 – Change in the morphometric parameters of alfalfa plants grown on oil-contaminated soil when introduced into the soil with the biopreparation "Lenoil" and spraying the plants with the biopreparations "Elena" and "Agrobiologist"

Experiment variant	Shoot length, cm	Root length, cm	Shoot mass, g	Root mass, g
PBN	$4,51^d \pm 0,34$	$2,51^c \pm 0,22$	$3,15^c \pm 0,26$	$1,55^c \pm 0,12$
PSN	$1,14^a \pm 0,09$	$0,84^a \pm 0,08$	$0,74^a \pm 0,01$	$0,64^a \pm 0,04$
PSN+L	$3,01^b \pm 0,22$	$1,86^b \pm 0,12$	$1,16^a \pm 0,08$	$0,76^b \pm 0,06$
PSN+ L+E	$4,23^d \pm 0,38$	$3,13^d \pm 0,28$	$4,32^d \pm 0,22$	$1,23^c \pm 0,08$
PSN+ L+Ag	$3,31^c \pm 0,28$	$2,31^c \pm 0,22$	$3,31^c \pm 0,34$	$1,03^b \pm 0,08$

Note: PBN – soil without oil; PSN – soil with oil; PSN + L - oil + Lenoil; PSN + L + E - oil + Lenoil + Elena; PSN + L + Ag - oil + Lenoil + Agrobiolog; n=5; mean values ± standard error is presented; significantly different means (p ≤ 0,05, t-test) for each indicator are marked with different letters

Comparing the effectiveness of oil degradation with the help of Lenoil biopreparations in terms of the activity of growth processes in alfalfa plants, it can be noted that when Lenoil biopreparations were used, the plants were somewhat inferior in morphometric parameters to the control without oil, but significantly differed from the control with oil.

The use of the biological product "Agrobiologist" for spraying to stimulate growth processes did not show a good growth-activating effect on alfalfa plants (Table 1).

Thus, the combination of two types of treatments (root and foliar) with microbiological preparations allowed rye and alfalfa plants to activate growth processes under conditions of growth on oil-contaminated soils. The natural ability of plants and rhizosphere microorganisms to degrade and accumulate various pollutants is known [17]. The content of accumulated pollutants in certain types of plants can be tens and hundreds of times higher than their content in the environment [3]. The role of microorganisms in the degradation of oil hydrocarbons is usually described in the literature [1], [2]. In our studies, we also carried out the initial treatment of oil-contaminated soil with biopreparations-oil destructors. However, there is little information in the literature on the role of phytoremediant plants in the phytostimulation of destructor microbes living in the root rhizosphere. Plants with an extensive root system secrete specific biologically active substances that promote the growth of colonies of microorganisms that activate the process of oil biodegradation and restore soil fertility [18], [19], [20], [21]. One of the indicators of the effectiveness of using the microbial-plant association for phytoremediation of soil contaminated with oil products can be the assessment of the state of some components of the plant's antioxidant system (Table 2).

Determination of the content of hydrogen peroxide in the roots of alfalfa plants showed that the highest concentration of H_2O_2 was observed in the variants with oil introduced into the soil and the biopreparation Lenoil (PSN+L) (Table 2).

It is known that the generation of reactive oxygen species, in particular changes in the level of H_2O_2 in plant cells under stress, is an indicator of oxidative stress and is used to transmit a signal in response to changes in environmental conditions [23]. Probably, the oxidative reaction activates plant defense mechanisms by increasing the activity of enzymes of the antioxidant system (catalase and a group of peroxidases) [24].

We established different levels of catalase enzyme activity, so in the “PSN + L + E” variant, the highest level of catalase activity was set, 3.5 more than in the PBN control, but in the “PSN + L” variant, catalase activity was 64% was higher than in the PBN control. In a number of works, it is noted that “the balance between the accumulation and repayment of H_2O_2 is of great importance for cell survival” and an increase in catalase activity may be associated with an increase in plant stress resistance [23]. In the values demonstrating the activity of the peroxidase enzyme, no significant differences were found between the control and experimental variants (Table 2).

It can be assumed that the relatively high content of superoxide anion in rye plants of the PSN, PSN+L, and PSN+L+E variants indicates insufficient activity of the superoxide dismutase enzyme (Table 2). Taking into account the relatively high content of hydrogen peroxide in the samples of the variants “PSN+L” and “PSN+L+E”, it can be assumed that in plants of these variants, the metabolic chain of ROS is actively functioning [23].

Some authors note that the activity of antioxidant enzymes (catalase, peroxidases, etc.) depends on the resistance of the plant to unfavorable environmental factors [10], [25]. In our case, taking into account the positive protective effect of biological preparations on plants growing on oil-contaminated soil, it can be assumed that the products of the interchange of plants and microorganisms, as well as a decrease in the toxic effect of oil products on the roots under the action of biological preparations, have a beneficial effect on plant growth.

Table 2 – Changes in the content of hydrogen peroxide, superoxide anion, peroxidase and catalase activity, in the roots of alfalfa plants grown on oil-contaminated soil when biopreparations "Lenoil" are introduced into the soil and plants are sprayed with biopreparations "Azolen" and "Agrobiologist"

Experience Variant	Content		Activity	
	H_2O_2, OE/g fresh weight	superoxidanion, concentration of adrenochrome, OE/g fresh weight	peroxidase, OE/g fresh weight	catalase, OE/g fresh weight
PBN	192,43^a±12,32	0,565^c±0,042	1,4^b±0,1	0,17^b±0,02
PSN	194,31^a±18,38	1,013^e±0,092	0,6^a±0,2	0,10^a±0,01
PSN+L	833,17^c±36,44	1,179^e±0,086	0,9^b±0,05	0,28^c±0,02
PSN+ L+E	950,37^d±66,24	1,507^f±0,058	1,7^c±0,3	0,58^d±0,05
PSN+ L+Ag	280,04^b±16,56	0,420^b±0,028	1,6^c±0,1	0,26^c±0,02

Note: PBN – soil without oil; PSN – soil with oil; PSN + L - oil + Lenoil; PSN + L + E - oil + Lenoil + Elena; PSN+L+Ag-neft+Lenoil+Agrobiologist; n=5; mean values ± standard error is presented; significantly different means ($p \leq 0,05$, t-test) for each indicator are marked with different letters

4. Conclusion

Thus, the combination of two types of treatments with microbiological preparations (root (Lenoil) and foliar (Elena or Agrobiologist) allowed alfalfa plants to activate growth processes under conditions of growth on oil-contaminated soils. it can be assumed that the products of the interchange of plants and microorganisms, as well as a decrease in the toxic effect of oil products on the roots under the action of biological preparations, have a beneficial effect on plant growth.

Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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