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Experimental Study of the Impact of *Alisma plantago-aquatica* Secretions on Pathogenic Bacteria

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Aim. To estimate the impact of *in vivo* secretions of water plantain (*Alisma plantago-aquatica*) on the populations of pathogenic bacteria *Erysipelothrix rhusiopathiae*. **Methods.** The plants were isolated from their natural conditions, the roots were washed from the substrate residues and cultivated in laboratory conditions for 10 days to heal the damage. Then the water was changed; seven days later the selected samples were sterilized using filters with 0.2 μm pore diameter. The dilution of water plantain root diffusates in the experimental samples was 1:10–1:10,000. The initial density of *E. rhusiopathiae* bacteria populations was the same for both experimental and control samples. The estimation of the results was conducted 48 hours later. **Results.** When the dilution of root diffusates was 1:10, the density of erysipelothrixes in the experimental samples was 11.26 times higher than that of the control, on average, the dilution of 1:100 − 6.16 times higher, 1:1000 – 3.22 times higher, 1:10,000 – 1.81 times higher, respectively. **Conclusions.** The plants of *A. plantago-aquatica* species are capable of affecting the populations of *E. rhusiopathiae* pathogenic bacteria via the secretion of biologically active substances into the environment. The consequences of this interaction are positive for the abovementioned bacteria, which is demonstrated by the increase in the density of their populations in the experiment compared to the control. The intensity of the stimulating effect on the populations of *E. rhusiopathiae* in the root diffusates of *A. plantago-aquatica* is reciprocally dependent on the degree of their dilution. The investigated impact of water plantain on erysipelothrixes should be related to the topical type of biocenotic connections, the formation of which between the test species in the ecosystems might promote maintaining the potential of natural focus of rabies.

Keywords: *Alisma plantago-aquatica, in vivo* secretions, *Erysipelothrix rhusiopathiae*, population density, topical type of connections.

INTRODUCTION

The capability of causal agents of some human and animal diseases, so called sapronoses, to exist both in natural and changed ecosystems has been proven in a number of scientific articles [1–3]. The specificity of these pathogenic microorganisms is their circulation in the biocenoses, the duration of existence, the density of populations and other properties of causal agents are of great epidemiological and epizootic relevance and are considerably determined by their interaction with other living components of ecosystems.

Plants are known for their significant role in the terraneous and aquatic biocenoses as they create the conditions for the existence of many living organisms [4], including pathogenic ones. The scientific literature contains the data about the impact of plants on such pathogenic microorganisms as Yersinia, vibrions, Salmonella, Leptospira, etc. [5–12].

Our study of the ecology of *Erysipelothrix rhusiopathiae* bacteria (Migula, 1900) – rabies agents [13] – did not reveal any articles published, describing the specificities of the interaction of these microorganisms and plants. Taking this fact into consideration the current work was aimed at the study and characterization of the impact specificities of the most wide-spread aquatic and coastal species of plants on the populations

of *E. rhusiopathiae* pathogenic bacteria. The results of the study of these interactions with some plant species have already been published [14–17]. At the same time, there is a number of rather wide-spread species of higher plants, the impact of which on the rabies agent has not been studied yet. One of these plants is water plantain (*Alisma plantago-aquatica* L. 1753) [18] – a perennial plant, growing on watersides, marshy meadows, grassy and sedge marshes in the whole territory of Ukraine [19].

MATERIALS AND METHODS

Pure cultures of erysipelothrixes were obtained from the collection of the Institute of Veterinary Medicine NAAS. *E. rhusiopathiae* bacteria (BP-2 IBM strain) were cultivated on the brain heart infusion broth (AES Chemunex, France) at 36.7 ± 0.3ºC for 48 h.

The samples of water plantain were selected in their typical habitat, dug round and extracted together with the balk of soil, containing short and massive roots of the plant. In laboratory conditions the soil was washed out with the water flow until complete baring of the root system. During the extraction, transportation and washing-out of soil these plants have got some damage, through which the substances, not secreted by the plant in natural conditions, may penetrate the aqueous solution. To heal the damage, the plant roots were placed into pure glass containers, filled with water from the water supply system, which was settled for two days. Ten days later the container was emptied and re-filled with fresh water. To prevent reoccurring damage the plants were not taken out and their position was not changed. The water level was maintained constantly, compensating its loss from evaporation and transpiration.

The root diffusates of *A. plantago-aquatica* were obtained by the method, described by Hrodzyns'kyy [20]. Seven days later the water samples for biotesting were selected from the containers where the plants were located in the conditions, similar to natural fluctuations of temperature and lighting. The experiments using pure cultures of microorganisms require the sterilization of the root diffusate solutions of water plantain. To prevent the dissolution and change of substances, aqueous solutions of the secretions of the investigated plant species were sterilized by vacuum filtration through the bacterial filters with the pore diameter of 0.2 μm.

The impact of *in vivo* secretions of water plantain on the populations of *E. rhusiopathiae* pathogenic bacteria was studied in several dilutions, which were 10 times different from each other, namely, 1:10, 1:100, 1:1000 and 1:10,000. The abovementioned dilutions of plant secretions were obtained by the addition of the sterilized water from the water supply system and 0.1 cm^3 of the bacterial culture of *E. rhusiopathiae*. The samples, containing similar ratios of water and bacterial culture, were used as a control. The initial density of the erysipelothrixes in the test and control samples was the same, as the samples for inoculation were selected from the same vial.

Both test and control samples were kept at the temperature of 20.0 ± 2.0 °C. The samples were selected 48 h later; a number of consecutive dilutions were pre-

The impact of root diffusates of *A. plantago-aquatica* on the density of populations of *E. rhusiopathiae* bacteria (P* ≤ 0.001)

No. of experiment	Density of *E. rhusiopathiae* populations, millions of cells/cm^3				
	Experiment (dilution of secretions)				Control
	1:10	1:100	1:1000	1:10 000	
1	29.90	17.20	9.30	5.30	2.30
2	30.20	16.70	8.40	4.80	2.50
3	29.70	17.10	9.00	5.00	3.10
4	32.30	16.50	8.80	5.20	2.40
5	31.70	16.90	8.50	4.70	3.20
6	32.00	17.30	9.20	4.90	3.00
M	30.97	16.95	8.87	4.98	2.75
For dilution 1:10			t = 51.56	t_{cr} = 4.59	
For dilution 1:100			t = 63.50	t_{cr} = 4.59	
For dilution 1:1000			t = 24.41	t_{cr} = 4.59	
For dilution 1:10000			t = 10.93	t_{cr} = 4.59	

Note. M – arithmetic mean; t – Student's coefficient; t_{cr} = critical value of the t index; P – probability degree.

pared therefrom, and sown in 0.1 cm^3 in Petri dishes on the surface of nutrient agar (AES Chemunex, France) to determine the density of erysipelothrixes. Petri dishes with the inoculations were cultivated in the thermostat for 72 h at 36.7 ± 0.3 °C. The number of colonies was calculated under the MBS-10 binocular microscope. The estimation of the mean number of living bacteria in the test and control samples per 1.0 cm^3 was performed using the mathematical methods.

RESULTS AND DISCUSSION

The capability of water plantain to affect the populations of *E. rhusiopathiae* pathogenic bacteria (*in vitro*) is vividly demonstrated by the results of the experiments (Table).

It is noteworthy that the density indices for erysipelothrixes in all the test samples were higher than those for the control. The probability of the differences, revealed in the density of cells, was proven by the statistical analysis. Therefore, it is possible to state that the root diffusates of water plantain in the investigated range of dilutions have stimulating impact on the populations of *E. rhusiopathiae* pathogenic bacteria.

It should also be noted that the difference between the density of cells in the test and control samples decreases with the increase in the dilution of solutions with plant secretions. For instance, when the dilution of root diffusates was 1:10, the density of erysipelothrixes in the experimental samples was 11.26 times higher than that of the control, on average, the dilution of 1:100 – 6.16 times higher, 1:1000 – 3.22 times higher, 1:10,000 – 1.81 times higher, respectively. The determination of the correlation degree between the value of the dilution index of root diffusates of water plantain and the density of *E. rhusiopathiae* bacteria cells demonstrated the inverse relationship of medium distinctiveness between them (correlation coefficient r = –0.67). In our opinion, the detected effect may be explained as follows. During the vegetation period water plantain plants actively secrete a complex of biologically active substances (BAS) through their roots into the environment (water); the mentioned substances have stimulating effect on the populations of *E. rhusiopathiae* bacteria, leading to the increase in the density of the latter. In the experiment conditions the maximal content of BAS, secreted by the plant, was registered in the samples with 1:10 ratio of the plant secretion dilution. We also noted the highest stimulating effect on the populations of the studied microorganism strain in the samples with this dilution index. The increase in the index of dilution resulted in the decrease in BAS concentration of water plantain which was demonstrated by the decrease in the positive impact on *E. rhusiopathiae* populations.

The data, obtained by us about the stimulating effect of water plantain secretions on the populations of *E. rhusiopathiae* pathogenic bacteria, testify to the fact that there may be beneficial conditions for the existence of erysipelothrixes in the habitat of this plant species in fresh waters and in coastal areas. The relationship, detected between the species, is related to the category of the topical type of biocenotic relations, as the presence of an *A. plantago-aquatica* plant is favorable for the conditions of existence of *E. rhusiopathiae* bacteria, and the latter react to this impact by the density change in their populations.

CONCLUSIONS

The plants of *A. plantago-aquatica* species are capable of affecting the populations of *E. rhusiopathiae* pathogenic bacteria via the secretion of biologically active substances into the environment. The consequences of this interaction are positive for the abovementioned bacteria, which is demonstrated by the increase in the density of their populations in the experiment compared to the control. The intensity of the stimulating effect on the populations of *E. rhusiopathiae* in the root diffusates of *A. plantago-aquatica* is reciprocally dependent on the degree of their dilution. The investigated impact of water plantain on erysipelothrixes should be related to the topical type of biocenotic connections, the formation of which between the test species in the ecosystems might promote maintaining the potential of natural focus of rabies.

The limited amount of the data on the existence conditions of the agents of natural focal diseases, in particular, *E. rhusiopathiae* bacteria, as well as their great epidemiological and epizootic relevance presuppose the significance of further study of their ecologic relations with different components of biocenoses.

Експериментальне вивчення впливу виділень *Alisma plantago-aquatica* на патогенних бактерій

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Мета. Оцінити вплив прижиттєвих виділень частухи подорожникової (*Alisma plantago-aquatica*) на популяції патогенних бактерій *Erysipelothrix rhusiopathiae*.

Методи. Рослини вилучали з природних умов, корені відмивали від залишків субстрату та культивували за лабораторних умов упродовж 10 діб для загоювання пошкоджень. Після цього воду змінювали, а через 7 діб відібрані проби стерилізували за допомогою фільтрів з діаметром пор 0,2 мкм. У дослідних зразках розведення кореневих дифузатів частухи подорожникової становили 1:10−1:10000. Початкова щільність популяцій бактерій *E. rhusiopathiae* у дослідних і контрольних зразках була однаковою. Через 48 год проводили облік результатів. **Результати.** В експериментальних зразках за розведення кореневих дифузатів 1:10 щільність клітин еризипелотриксів була в середньому в 11,26 раза вищою, ніж у контролі, за розведення виділень 1:100 − у 6,16 раза, 1:1000 – у 3,22 раза, 1:10000 – в 1,81 раза. **Висновки.** Рослини виду *A. plantago-aquatica* через виділення у середовище існування біологічно активних речовин здатні впливати на популяції патогенних бактерій *E. rhusiopathiae*. Для останніх наслідки таких взаємодій є позитивними, що проявляється у збільшенні щільності їхніх популяцій у досліді порівняно з контролем. Інтенсивність стимулювального впливу на популяції *E. rhusiopathiae* кореневих дифузатів *A. plantago-aquatica* у зворотно залежить від ступеня їхнього розведення. Досліджений вплив частухи подорожникової на еризипелотриксів слід віднести до топічного типу біоценотичних зв'язків, формування якого між піддослідними видами в екосистемах, ймовірно, сприяє підтриманню потенціалу природних вогнищ бешихи.

Ключові слова: *Alisma plantago-aquatica,* прижиттєві виділення, *Erysipelothrix rhusiopathiae*, *щільність популяцій, топічний тип зв'язків.*

Экспериментальное изучение влияния выделений *Alisma plantago-aquatica* на патогенных бактерий

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Цель. Оценить влияние прижизненных выделений частухи подорожниковой (*Alisma plantago-aquatica*) на популяции патогенных бактерий *Erysipelothrix rhusiopathiae*. **Методы.** Растения изымали из природных условий, корни отмывали от остатков субстрата и культивировали в лабораторных условиях на протяжении 10 сут для заживления повреждений. После этого воду меняли, а через 7 сут отобранные образцы стерилизовали з помощью фильтров с диаметром пор 0,2 мкм. В опытных образцах разведения корневых диффузатов частухи подорожниковой составляли 1:10−1:10000. Начальная плотность популяций бактерий *E. rhusiopathiae* в опытных и контрольных образцах была одинаковой. Через 48 ч проводили учет результатов. **Результаты.** В опытных образцах при разведении корневых диффузатов 1:10 плотность популяций эризипелотриксов была в среднем в 11,26 раза выше, чем в контроле, при разведении 1:100 – в 6,16 раза, 1:1000 – в 3,22 раза, 1:10000 – в 1,81 раза. **Выводы.** Растения вида *A. plantago-aquatica* через выделение в среду обитания биологически активных веществ способны влиять на популяции патогенных бактерий *E. rhusiopathiae*. Для последних последствия этих взаимодействий положительны, что проявляется в увеличении плотности их популяций в опыте по сравнению с контролем. Интенсивность стимулирующего эффекта на популяции *E. rhusiopathiae* корневых диффузатов *A. plantago-aquatica* находится в обратной зависимости от степени их разведения. Исследован-ное действие частухи подорожниковой на эризипело-триксов следует отнести к топическому типу биоценотических связей, формирование которых между подопытными видами в экосистемах, вероятно, способствует поддержанию потенциала природных очагов рожи.

Ключевые слова: *Alisma plantago-aquatica,* прижизненные выделения, *Erysipelothrix rhusiopathiae*, плотность популяций, топический тип связей.

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