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REACTION OF THE POPULATIONS OF DISEASE CAUSATIVE AGENTS IN ANIMALS TO THE INFLUENCE OF *SAGITTARIA SAGITTIFOLIA L.* INTRAVITAL EXCRETIONS

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Outbreaks of infectious diseases, such as erysipelas and leptospirosis, which cause significant economic damage to businesses and households, are registered in Ukraine annually. The ability of pathogen agents of these diseases (Erysipelothrix rhusiopathiae bacteria and Leptospira interrogans spirochaetes) to survive in the environment objects and circulate in ecosystems for a long time makes fighting against these infectious agents and disease prevention extremely difficult and costly.

The density of population and the duration of causative agents of infections in the environment objects are significantly influenced by biotic factors and plants in particular, which are a powerful source of biologically active substances in the ecosystem.

It has been established by the research that root diffusates of arrowhead Sagittaria sagittifolia have a stimulating effect on the population of E. rhusiopathiae bacteria when diluted in 1:10 and 1:100 proportions. Erysipelothrix density in the experimental samples was higher at 177.86 % and 59.87 % respectively than in the control sample. When diluted in 1:1000 and 1:10000 proportions, intravital excretions of arrowhead did not have a clear impact on the populations of erysipelas causative agent.

The experiments, studying the influence of arrowhead root diffusates on the density of the populations of leptospirosis pathogens, were conducted on the cultures of 7 serological strains of L. interrogans. The analysis of the results showed that intravital excretions of arrowhead have a vivid depressing effect on the experimental leptospira cultures. Serological variants of L. interrogans differed by the degree of sensitivity to the influence of S. sagittifolia excretions, and the rate of inhibition of spirochaetes populations ranged from 25.53 % to 82.86 %.

In the conditions of coastal and water ecosystems, S. sagittifolia plants demonstrate an allelopathic reaction to the populations of pathogenic E. rhusiopathiae and L. interrogans bacteria, resulting in the topical type of ecological relations between these species.

Keywords: *SAGITTARIA SAGITTIFOLIA*, ROOT DIFFUSATES, *ERYSIPELOTHRIX RHUSIOPATHIAE LEPTOSPIRA INTERROGANS,* TOPICAL RELATIONS

РЕАКЦІЯ ПОПУЛЯЦІЙ ЗБУДНИКІВ ЗАХВОРЮВАНЬ ТВАРИН НА ВПЛИВ ПРИЖИТТЄВИХ ВИДІЛЕНЬ *SAGITTARIA SAGITTIFOLIA L.*

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На території України щорічно реєструються спалахи таких інфекційних хвороб як бешиха та лептоспіроз, що завдає значної економічної шкоди підприємствам та господарствам. Здатність збудників цих хвороб бактерій Erysipelothrix rhusiopathiae та спірохет Leptospira interrogans

тривалий час зберігатись в об'єктах зовнішнього середовища та циркулювати в екосистемах робить боротьбу з цими інфекційними агентами та профілактику захворювань вкрай складною та затратною.

На щільність популяцій та тривалість існування збудників інфекцій в об'єктах зовнішнього середовища суттєвий вплив здійснюють біотичні фактори, зокрема рослини, які є потужним джерелом надходження в екосистеми біологічно активних речовин.

Дослідженнями встановлено, що кореневі дифузати стрілолиста стрілолистого (Sagittaria sagittifolia) у розведеннях 1:10 та 1:100 здійснюють стимулюючий вплив на популяції бактерій E. rhusiopathiae. Щільність еризипелотріксів у дослідних зразках була відповідно на 177,86 % та 59,87 % вищою ніж у контролі. У розведеннях 1:1000 та 1:10000 прижиттєві виділення стрілолиста не здійснювали виразного впливу на популяції збудника бешихи.

В експериментах із вивчення впливу кореневих дифузатів стрілолиста на щільність популяцій збудників лептоспірозів тестування проводили із культурами 7 серологічних штамів L. interrogans. Аналіз одержаних результатів показав, що прижиттєві виділення стрілолиста здійснюють виразний пригнічуючий вплив на піддослідні культури лептоспір. Серологічні варіанти L. interrogans відрізнялись між собою за ступінню чутливості до впливу з боку виділень S. sagittifolia, а показник пригнічення популяцій спірохет коливався в межах від 25,53 % до 82,86 %.

В умовах прибережних та водних екосистем рослини S. sagittifolia виявляють алелопатичну активність по відношенню до популяцій патогенних бактерій E. rhusiopathiae та L. interrogans, в результаті чого між цими видами формується топічний тип екологічних взаємозв'язків.

Ключові слова: *SAGITTARIA SAGITTIFOLIA*, КОРЕНЕВІ ДИФУЗАТИ, *ERYSIPELOTHRIX RHUSIOPATHIAE*, *LEPTOSPIRA INTERROGANS*, ТОПІЧНІ ВЗАЄМОЗВ'ЯЗКИ

РЕАКЦИЯ ПОПУЛЯЦИЙ ВОЗБУДИТЕЛЕЙ ЗАБОЛЕВАНИЙ ЖИВОТНЫХ НА ВЛИЯНИЕ ПРИЖИЗНЕННЫХ ВЫДЕЛЕНИЙ *SAGITTARIA SAGITTIFOLIA L.*

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На территории Украины ежегодно регистрируются очаги таких инфекционных болезней как рожа и лептоспироз, которые наносят существенный экономический вред предприятиям и хозяйствам. Способность возбудителей этих болезней бактерий Erysipelothrix rhusiopathiae и спирохет Leptospira interrogans длительное время сохранятся в объектах внешней среды и циркулировать в экосистемах делает борьбу с этими инфекционными агентами и профилактику заболеваний крайне сложной и затратной.

На плотность популяций и длительность существования возбудителей инфекций в объектах внешней среды существенное влияние оказывают биотические факторы, в частности растения, которые являются мощным источником поступления в экосистемы биологически активных веществ.

Исследованиями показано, что корневые выделения стрелолиста стрелолистого (Sagittaria sagittifolia) в разведениях 1:10 и 1:100 осуществляют стимулирующее воздействие на популяции бактерий E. rhusiopathiae. Плотность эризипелотриксов в опытных образцах была соответственно на 177,86 % и 59,87 % выше чем в контроле. В разведениях 1:1000 и 1:10000

прижизненные выделения стрелолиста не оказывали выраженного воздействия на популяции возбудителей рожи.

В экспериментах по изучению влияния корневых выделений стрелолиста на плотность популяций возбудителей лептоспирозов тестирование проводили с культурами 7 серологических штаммов L. interrogans. Анализ полученных результатов показал, что прижизненные выделения стрелолиста оказывают выраженное угнетающее воздействие на подопытные культуры лептоспир. Серологические варианты L. interrogans отличались между собой по чувствительности к влиянию со стороны выделений S. sagittifolia, а показатель угнетения популяций спирохет колебался от 25,53 % до 82,86 %.

В условиях прибрежных и водных экосистем растения S. sagittifolia выявляют алеллопатическую активность по отношению к популяциям патогенных бактерий E. rhusiopathiae и L. interrogans в результате чего между этими видами формируется топический тип экологических взаимосвязей.

Ключевые слова: *SAGITTARIA SAGITTIFOLIA*, КОРНЕВЫЕ ДИФУЗАТЫ, *ERYSIPELOTHRIX RHUSIOPATHIAE*, *LEPTOSPIRA INTERROGANS*, ТОПИЧЕСКИЕ ВЗАИМОСВЯЗИ

The ability of some causative agents of infections to exist in the composition of natural and anthropologically changed ecosystems for a long time is a well-accepted truth [1].

However, the mechanisms and ways of circulation of these infectious agents have not been studied enough. At the same time, the duration of life and the population density of pathogenic organisms in the environment objects are of great practical importance, as they are capable of determining to a large extent the possibility of human and animal contamination on certain territories.

The localizations of such diseases as erysipelas and leptospirozis are registered in Ukraine annually. The infectious agents of these diseases — *Erysipelothrix rhusiopathiae* bacteria (Migula, 1900) and spirochaete *Leptospira interrogans* (Stimson, 1907, Wenyon, 1926) are capable of long-term circulation on the territories of the so-called «natural disease locations», posing a constant threat of infection to human beings, domestic and agricultural animals [2–5].

The duration of infectious agents circulation under specific conditions is determined by a range of factors, influencing pathogenic organisms directly and indirectly. As pointed out by the following works [6–8], biotic factors are able to have a considerable influence on the density dynamics of the pathogenic microorganisms in the environment objects as well as on their characteristics.

High plants are known to play an important environment-forming role in land and water ecosystems, determining the living conditions of a number of organisms to a considerable degree [9, 10], including such pathogenic agents as *erysipelothrix* and *leptospira*.

Material and methods

The influence of arrowhead root diffusions (*Sagittaria sagittifolia L.)* on the pathogenic bacteria population density of *E. rhusiopathiae* and *L. interrogans* spirochaeta has been studied.

The samples of arrowhead were picked from their natural growing stations. The substratum was washed off their roots, and the plants were placed into plastic reservoirs filled with cold tap water. In a 5-day period, after the root injuries had been healed, the water was changed completely. The plant mass and water concentration was 1:10. Root diffusates exposition lasted for 7 days and took place under natural variations of light and day and night temperature. The obtained solution of *S. sagittifolia* excretions was sterilized with the help of bacterial cellulose filters with the pore diameter of 0.2 micrometres.

Test species of *E. rhusiopathiae* bacteria were grown on the heart and brain broth (AES Chemunex, France) at the

temperature of 36.7±0.3 ºC during 48 hours. Experimental samples contained the solutions of root diffusates in the concentration of 1:10, 1:100, 1:1000 and the quantity of 0.9 cm^3 to which 0.1 cm^3 of *E. rhusiopathiae* bacteria were added. Control samples contained analogical correlation between sterilized tap water and *erysipelothrixes*. The experiment was repeatedly conducted six times. Experimental and control samples were kept at the indoor temperature (18...20 ºC) for 48 hours. The *erysipelothrix* population density was calculated by means of 0.1 cm^3 sample planting in the subsequent diffusions of $1x10^{-3}$ and $1x10^{-4}$ on the heart and brain broth (AES Chemunex, France) in three Petri dishes, and were cultivated under the temperature (36.7±0.3 ºC) for 72 hours with the following calculation of colonies that have grown, and estimating the average quantity of living bacteria per $1cm^3$.

Test species of *L. interrogans* spirochaeta were grown with the help of the Ters'ky medium containing 5% of rabbit blood serum at the temperature of 28.0±0.3 ºC. Diagnostic strains of leptospiras were used in the experiment (tabl. 1).

Table 1

The list of *Leptospira interrogans* stems used in the experiments

№	Serologycal group	Serologycal variant	Stems	Conditional reduction
1	Sejroe	pollonica	493 Poland	*L. pollonica*
2	Hebdomadis	kabura	Kabura	*L. kabura*
3	Tarassovi	tarassovi	Perepelicyn	*L. tarassovi*
4	Pomona	pomona	Pomona	*L. pomona*
5	Grippotyphosa	grippotyphosa	Moskva V	*L. grippotyphosa*
6	Canicola	canicola	Hond Utrecht IV	*L.canicola*
7	Icterohaemorrhagiae	copenhageni	M 20	*L. icterohaemorrhagiae*

During the experiments aimed at investigating the influence of *S. sagittifolia* root diffusates on the *L. interrogans* spirochaeta, the test-tubes were filled with 0.4 cm^3 of the obtained test solution and 0.1 cm^3 of leptospira species. Control samples were based on the analogical correlation of the distilled water and leptospira species. Inoculates for test and experimental samples were selected from one and the same mother plant. Thus, the initial quantity of leptospira in the tests was the same. Leptospira species density was calculated 24 hours after the beginning of the experiment by means of direct counting of a known volume of leptospira with the help of Samostrelsky's method [11].

Results and discussion

The results of the study showed that the density of erysipelothrixes in the experimental samples was 177.86 % higher than in the control samples in a small dilution (1:10), i.e. root excretions of *S. sagittifolia* caused quite a strong reproduction stimulation in the populations of *E. rhusiopathiae* bacteria (tabl. 2).

The stimulating effect of *S. sagittifolia* root excretions was also observed in the samples with diffusates dilution 1:100. The density of erysipelothrixes in the experimental samples was higher by 59.87 % than in the control samples.

A similar effect was not observed in case of higher levels of dilution (1:1000 and 1:10 000). The statistic calculation of the experiment results demonstrates that the difference in the density of erysipelothrixes in the experimental and control samples with 1:1000 and 1:10000 dilutions of *S. sagittifolia* root excretions is not valid. Experimental results show that in moderate and big concentrations, intravital excretions of *S. sagittifolia* do not have a considerable influence on the population density of *E. rhusiopathiae* bacteria.

Table 2

***E. rhusiopathiae* cell density comparison in experimental and control samples under the influence of *S. sagittifolia* root diffusates**

Number of experiment	*E. rhusiopathiae* cell density, million / cm^3				
	Experiment dilution of excretions				Control
	1:10	***1:100***	***1:1000***	***1:10 000***	
1	5.60	4.30	2.80	2.50	3.00
2	7.10	3.80	2.30	2.10	2.40
3	8.30	4.00	2.20	2.00	3.50
4	7.10	3.90	2.65	2.40	2.00
5	6.00	4.20	2.70	2.60	2.10
6	8.10	4.10	3.00	2.30	2.20
M*	**7.03**	**4.05**	**2.61**	**2.32**	**2.53**
For dilution 1:10	t = 8.15 at	t_{cr}= 4.59;	P = 0.001		
For dilution 1:100	t = 5.46 at	t_{cr}= 4.59;	P = 0.001		
For dilution 1:1000	t = 0.25 at	t_{cr}= 4.59;	P = 0.001		
For dilution 1:10 000	t = 0.76 at	t_{cr}= 4.59;	P = 0.001		

Note: Henceforth: M — arithmetical mean; t — coefficient of Student; t_{cr} — critical meaning of t parameter; P — level of probability

The data obtained in the course of the experiments studying the influence of *S. sagittifolia* root excretions on the populations of serological variants of leptospira are provided in table 3.

Table 3

***L. interrogans* cell density comparison in experimental and control samples under the influence of *S. sagittifolia* root diffusates**

№	*L. interrogans* cell density, million / cm^3							
	L. pollonica		*L. kabura*		*L. canicola*		*L. pomona*	
	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
1	6.97	9.66	3.30	4.88	4.17	9.62	1.44	3.86
2	6.58	9.38	3.43	4.53	4.00	9.10	1.41	4.32
3	7.05	9.65	3.57	4.67	3.82	9.23	1.38	3.97
4	6.64	9.74	3.62	4.64	4.37	9.74	1.40	4.17
5	6.76	9.57	3.58	4.78	4.14	9.56	1.37	4.43
M	6.80	9.60	3.50	4.70	4.10	9.45	1.40	4.15
t	22.77		12.70		31.42		23.08	
t_{cr} = 5.04; P = 0.001								
	L. grippotyphosa		*L. icterohaemorrhagiae*				*L. tarassovi*	
	Experiment	Control	Experiment	Control			Experiment	Control
1	1,00	5,32	2,93	4,45			0,92	2,37
2	0,71	5,17	3,36	4,63			1,03	2,23
3	0,84	5,46	3,23	5,00			0,78	1,96
4	1,03	5,03	3,17	4,67			0,85	2,00
5	0,92	5,27	3,06	4,75			0,67	2,19
M	0,90	5,25	3,15	4,70			0,85	2,15
t	42,05		11,97				11,94	
$t_{кр}$= 5,04; P = 0,001								

High level of oppression influenced by root excretions of *S. sagittifolia* was noticed in the species of *L. grippotyphosa* (inhibition indicator was 82.86 %), *L. pomona* (66.27 %), *L. tarassovi* (60.47 %) and *L. canicola* (56.61 %). The populations of other serological variants of leptospira, such as *L. icterohaemorrhagiae* (inhibition indicator

32.97 %), *L. pollonica* (29.16 %), *L. kabura* (25.53 %*)* were oppressed in a smaller degree. Thus, the influence of *S. sagittifolia* root excretions on the populations of *L. interrogans* has a noticeable negative influence.

Under natural conditions and in the locations of such diseases as erysipelas and leptospirosis, excretions of various components of phytocenoses, *S. sagittifolia* in particular, have a considerable influence on the dynamics of the pathogenic bacteria population density in the environment objects.

Conclusions

1. In the conditions of coastal and water ecosystems, the plants of *S. sagittifolia* show allelopathic activity in relation to pathogenic bacteria populations of *E. rhusiopathiae* and *L. interrogans*. As a result, a topical type of ecological relations is formed between these two species

2. Root excretions of *S. sagittifolia* have a stimulating influence on the populations of *E. rhusiopathiae* only in case of small dilutions (1:10 and 1:100). If the concentration of *S. sagittifolia* excretions is reduced, the stimulating effect on the bacteria populations disappears.

3. The populations of *L. interrogans* are oppressed under the influence of intravital root excretions of *S. sagittifolia.* The inhibition indicator of the species was different in the observed serological groups of leptospira and varied from 25.53 % до 82.86 %.

The perspectives of further research. The data about the peculiarities of ecological interrelations between pathogenic bacteria and ecosystem components present interest for further working out of the effective methods of disease prevention among human beings and animals and sanation of the environment objects. It is necessary to continue an overall detailed investigation of topical relations between infectious agents and background species of phytocenoses in land and water ecosystems.

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