

Research Article

Plant molluscicide based on Smolevka white (*Silene Latifolia*) as prevention of pastoral helminthiasis of animals

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Abstract

Soft-bodied is the intermediate host of helminthiasis, in the body of which several development stages of larval forms of helminths occur. There is the highest population density of mollusks in the areas of ruminant grazing, which leads to mass infection of animals with trematodes. To destroy the intermediate host of helminths in agricultural production, molluscicidal remedies of synthetic and plant origin are used. The work aimed to determine the molluscicidal effectiveness of a plant remedy based on *Silene Latifolia* in conditions of natural pastures. The material for work was the green mass of the plant *S. Latifolia* obtained in the warm season from roots, leaves, stems, flowers, and seeds. By grinding this plant, a powder with a particle size of 1-3 mm was obtained. Then, the powder was extracted with ethyl alcohol. The obtained product (concentrate) was an amorphous gel-like mass of dark green color with a specific smell and well soluble in water. Fieldwork in natural pastures was carried out on 5 biotopes with an area of 4-25 m². Three species of gastropods were recorded from freshwater mollusks in the biotopes: *Planorbis planorbis*, *Planorbarius corneus*, *Physa fontinalis*, *Lymnaea truncatula*, and *L. palustris*. The results of experiments conducted in the conditions of pastures indicate a high molluscicidal activity of the studied plant agent on pond fish, intermediate hosts of trematodes pathogens. The effectiveness of the developed molluscicide on gastropods, when treated with a working solution (10.0 g/l) is from 98.1 to 100%.

Introduction

Helminthiasis are common in most animals and birds; they cause significant economic damage to agriculture. Parasitic diseases, especially pasture, in the biological development cycle have intermediate hosts - insect larvae, crustaceans, fish, reptiles, including mollusks. Mollusks are the sources of most trematodes and nematodes, invasions of living objects. In the body of freshwater mollusks, several stages of development (transformations) of the larval forms of these helminths (parthenogenesis, molting) occur.

Mollusks are resistant to environmental factors due to the thickness of the shimmering layer of the cover epithelium and the density of collagen fibers that form the skin-muscle sac of the invertebrate. Treatment against mollusks is carried out during the period of the active state of mollusks (caving, their development, and growth) at ambient temperature not lower than 13 °C and pH not higher than 7.6 units. Before the destination of flood meadows (floodplains), fertilizer application is stopped to evaluate the effectiveness of measures.

There are many ways to destroy grazing mollusks. Their use depends on the features of the biological cycle of mollusks (ecology, application conditions). In production, it is often used - spraying, pollinating, sowing, or scattering poisoned decoys, introducing molluscicides into a stream of water replenishing the reservoir, using aerosols, fumigation, and added surfaces (impregnation).

Molluscicides of chemical origin (compounds of copper, arsenic, zinc, barium, mercury, and polychloroterpenes, alcohols, phenols) are promising, but their use in large areas is relatively expensive. Such preparations are limited in availability and are toxic to the environment. For example, salicylanilides cause 100% death of lower crustaceans, and fish, and 65% - 84% of benthos die in the first hours after application to ponds [13]. Most chemical compounds have high molluscicidal activity; however, for their use in practical conditions, reliable safety is needed for humans, animals, birds, plants, and other microorganisms. It is important that at a certain time after treatment with molluscicides, the residual amount of chemicals contained in agricultural

More Information

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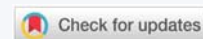
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Keywords: Extract; Freshwater mollusks; Molluscicide; Saponins





products obtained from the treated area is minimal and their concentration is safe for animals, and humans.

Preparations of plant origin are environmentally friendly and, therefore, are a little toxic to mammals, reptiles, amphibians, hydrations, and vegetation. This group of molluscicides is inexpensive, relatively accessible, and safe to use.

Drema white or Smolevka white (lat. *Silene Latifolia*) is a herbaceous dioecious plant of the genus Smolevok of the Clove family, growing in most countries of Europe, western Asia, and North Africa, a one- or biennial (sometimes perennial) plant 40-80 cm high. It contains a large number of saponins that are used in the production of medical and hygienic remedies [1,2].

In folk medicine, Smolevka white is valued for its saturated chemical composition [3-5]. In the underground and above-ground parts of the plant, there are tannins, flavonoids, synapic acid, triterpene glycosides (saponins), organic acids, resins, coumarin, homofientin, ferulic acid, vitamin C, alkaloids, isovitexin, orientin. Smolevka preparations are used to relieve mental stress in the form of baths and inside [3,5,6]. Decoctions and infusions of leaves and flowers are used as calming agents for epilepsy, and uterine bleeding, for the treatment of umbilical hernia inside and externally in the form of compresses. In the modern literature, there is evidence that Drema white is used in various tumor diseases of the intestine [7,8]. Seed tincture is used in neoplasms of the rectum and colon. In the experiment, preparations of the aboveground part of the plant increase the contractility of the uterine muscles and have anti-inflammatory and bile effects. Roots have a high foaming property and can be used to produce vegetable soap. Smolevka white is a valuable feed for small and large livestock [9-11]. Since the preparations of the *Silene Latifolia* plant contain saponins, they can have a molluscicidal property.

The purpose of this work was to determine the molluscicidal effectiveness of a plant remedy based on *S. Latifolia* against freshwater gastropod molluscs in laboratory conditions and natural pastures and to expand the natural raw material base for the production of drugs that reduce the number of gastropods.

Materials and methods

Preliminary work under laboratory conditions was carried out at the Federal State Budget Scientific Institution "Federal Scientific Centre VIEV", on artificial biotopes for invertebrates [12]. Studies were carried out on "pure" and invaded soft-bodied populations - *Lymnaea truncatula* (small pond) and *L. palustris* (swamp pond) by cultures of miracidia of the *Fasciola hepatica* pathogen. Mollusk viability was detected by microscopy under a Levenhuk 3ST binocular microscope while heating invertebrates on the Morozov table for 5-10 minutes on motor activity. We took into account the

presence of disturbances in normal processes of vital activity in mollusks (swelling, dehydration - the release of mucus, hemorrhagia) [13].

Production of molluscicidal remedy

The plant was harvested in the seasonal period of the year from July to October in the months 2018-2020 to achieve the maximum yield of the vegetation mass. At this time, in the physiology of the plant, flowering, and ripening of seeds occurs, including the accumulation of AVs (surfactants) and tannins. The plants were dug with roots, washed in tap water from the soil, and dried in shade without sunlight access to 13% - 15% humidity. Powder with biologically active substances of preparation from roots, leaves, stems, flowers, and seeds of the plant was prepared by grinding dried harvest in a mortar with a pestle to the size of 1-3 mm particles. A 2.5 g of plant raw material (powder) from the *S. Latifolia* plant was placed in a 50 ml beaker and 25 ml of distilled water was added. The cup was mounted on a magnetic stirrer with an anchor for 15 seconds at 150 rpm. The persistent foam formed on the surface of the slurry indicates the presence of surfactants in the prepared aqueous solution [5,13].

The prepared plant powder was placed in a 5 l beaker to which n-butanol was added at a rate of 1 g per 100 ml of liquid. Mixing of the plant slurry was carried out with a top-driven magnetic stirrer at 100 rpm. Extraction was carried out for 24 hours at a mixture temperature of 30 ± 2 °C. The extract was separated by a THERMO centrifuge (USA) at 8000 rpm. within 180 seconds. The supernatant was concentrated in a HEIDOLPH rotary evaporator (Germany) at a temperature of 60 ± 2 °C at 280 ± 5 rpm, as well as an air pressure of 4 kPa. The concentrate was further dried in a vacuum desiccator at room temperature and at a pressure of 10 kPa for 24 hours. The extractable substance was an amorphous gel-like mass of dark green color with a specific fine odor, highly soluble in water and alcohol. A working dilution of the remedy in the form of a 1% solution on tap water was prepared and biotopes were sprayed with mollusk populations.

Preparation of mollusks in the laboratory

To conduct the experiment in the laboratory in 2016-2017, wild populations of mollusks were brought and adapted [12]. The density of gastropods (*L. truncatula*, *L. palustris*) in laboratory cuvettes was 84-317 specimens per one m² (Test №1). The soil temperature was 18 ± 2 °C, and the pH of the medium was 6.7-7.0 units. Laboratory populations of soft-bodied were free of larval forms of helminths. In test №2, the population of a small pond was invaded by the miracidia of trematode *F. hepatica* in a dose of two specimens per 1st invertebrate. Processing molluscicidal remedy was administered 7-10 days after infection with trematodes. The working solution of *S. Latifolia* plant extract was used at a concentration of 1.0% (Table 1). The obtained aqueous solution of the remedy was sprayed with a Grida hand

sprayer (China) at a rate of 100 ml per one m² of artificial biotope area. As control № 1, a working solution of the plant extract *Saponaria Officinalis* [14] was used, and control №2 was served by two biotope areas with infected *F. hepatica* (*L. truncatula*) and "pure" (*L. palustris*) mollusk populations [12]. The control group of mollusks No. 2 was not treated with any drugs.

Use of mollusks in natural biotopes

The molluscicidal activity of the extract based on Smolevka white was carried out in the natural biotopes of the Ryazan region (Table 2). The production test was carried out for 7 days. Working remedies were dispersed with a 1000 ml manual Grida sprayer at the rate of 100 ml per one m² of the artificial biotope area. The objects for research were mollusks populations (*L. truncatula*, *L. palustris*, *Physa fontinalis*), coils (*Planorbis planorbis*, *Planorbarius corneus*), false leeches (*Haemopsis sanguisuga*), and lake frogs (*Pelophylax ridibundus*). In the spring, 5 biotopes were chosen (with an area of 4 to 25 m²) with mollusks on a pasture with a total area of 18 hectares. The distance of biotopes from each other was 50-250 m. The density of the natural planting of invertebrates in the studied areas was different: *L. truncatula* - from 11 to 157 specimens/m², *L. palustris* - from 24 to 204 specimens/m², *P. fontinalis* - from 13 to 19 specimens/m², *P. planorbis* - from 14 to 73

specimens/m², *P. corneus* - from 1 to 54 specimens/m². The water temperature was measured with an alcohol technical thermometer TTG-M (0-200°C) (Russia) and the soil reaction with a pocket pH meter Checker 1 HANNA (China). Processing with a molluscicide remedy based on a 1% working solution of *S. Latifolia* plant extract was carried out by spraying from the ZHUK apparatus OP-209 (Russia) with a volume of 9 liters at a rate of 100 ml per m² of the biotope. In biotope №1, an extract of the plant *S. Latifolia* was used, obtained from the underground part of the plant (rhizomes, roots), in biotope №2 from the aboveground part (stems, leaves, inflorescences, fruits). As control No. 1, a 1.0% aqueous solution of *S. Officinalis* plant extract was used (flow rate was 100 ml per m²). In control №2, a 0.2% working solution of copper sulfate was used (preparation consumption was 2 g per m²). In control №3, the use of molluscicide remedy was not carried out. The results of the study were taken into account 8 h, 24 h, and 7 days after the beginning of the experiment according to the method of V.V. Gorokhov and V.S. Osetrov (1987) [13].

Toxicological research

The study of toxic LD₅₀, LD₁₀₀ (in 30 white mice 2.5-3 months old with a mass of males and females 25 - 26 g and 30 white Wistar rats of both sexes 180 - 200 g) and cumulative K_{cuM} (on 10 white rats Wistar, males weighing 180 - 210 g)

Table 1: Molluscicidal efficiency of the 1% working solution of the product based on *Silene latifolia* in 8 hours after processing of mollusks in the laboratory.

Experience groups	Mollusc species	Working solution flow rate (ml/m ²)	Density of mollusks (specimens per 1 m ²)	Viable mollusks (specimens)	Viable molluscs (%)	
Test № 1	<i>L. truncatula</i>	100	291	0	0	
	<i>L. palustris</i>		185	2	1.1	
A _{average} ± σ _{error average}			238 ± 52	1 ± 1	0.6 ± 0.8	
Test № 2	<i>L. truncatula</i>		170	0	0	
Control № 1	<i>L. truncatula</i>		203	0	0	
	<i>L. palustris</i>		317	1	0.3	
A _{average} ± σ _{error average}			260 ± 81	0.5 ± 0.7	0.15 ± 0.15	
Control № 2	<i>L. truncatula</i>		-	129	98	75.9
	<i>L. palustris</i>			84	84	100
A _{average} ± σ _{error average}				107 ± 32	91 ± 9.9	87.9 ± 12.1

Table 2: The molluscicidal activity of the extract based on *Silene latifolia* (floodplain of the Ibrecki river of the Ryazan region of the Russian Federation, 27.05.2021, t water: 16.4 - 17.8°C, pH 6.5 - 7.1).

№ biotope	Mollusc species	Molluscicidal remedy (working solution concentration, flow rate)	Dead gastropods	Efficiency (%)	
1	<i>L. truncatula</i> -157, <i>P. planorbis</i> -73, <i>P. corneus</i> -54	<i>S. latifolia</i> extract (1% aqueous solution, 100 ml/m ²)	<i>L. truncatula</i> -155, <i>P. planorbis</i> -73, <i>P. corneus</i> -54	98.7 100 98.1	
			A _{average} ± σ _{error average}	94 ± 55	94 ± 53.7
2	<i>L. truncatula</i> -31, <i>L. palustris</i> -204		<i>L. truncatula</i> -31, <i>L. palustris</i> -201	100 98.5	
			A _{average} ± σ _{error average}		
3 (control № 1)	<i>L. truncatula</i> -122, <i>P. fontinalis</i> -13		<i>S. officinalis</i> extract (1% aqueous solution, 100 ml/m ²)	<i>L. truncatula</i> -120, <i>P. fontinalis</i> -13	98.3 100
		A _{average} ± σ _{error average}		67 ± 77.1	66 ± 75.7
4 (control № 2)	<i>P. fontinalis</i> -20, <i>P. planorbis</i> -89	Copper cuprous (0.2% aqueous solution, 2 g/m ²)		<i>P. fontinalis</i> -20, <i>P. planorbis</i> -89	100 100
				A _{average} ± σ _{error average}	54.5 ± 34.5
5 (control № 3)	<i>L. truncatula</i> -11, <i>L. palustris</i> -24, <i>P. planorbis</i> -14, <i>P. corneus</i> -1			-	<i>L. truncatula</i> -0, <i>L. palustris</i> -0, <i>P. planorbis</i> -0, <i>P. corneus</i> -0
			A _{average} ± σ _{error average}		12.5 ± 9.5



properties of the molluscicide from *S. Latifolia* was performed using standard methods [13,15-18].

Statistical processing

Statistical data processing was performed by determining the statistical significance of differences in average values according to the Student t-criterion using the applied computer program "Statistica 6".

Results

Preliminary studies conducted in the laboratory have shown 98.9-100% effectiveness of the alcohol extract of *S. Latifolia* (Table 1). The molluscicide remedy was effective at 1% strength dilution in tap water. During the first 10-15 minutes, gastropods showed high motor activity, and they released copious amounts of mucus from under their shells. Then, their activity moved to the stage of immobility (inhibition). After 8 hours, no viable mollusks were found.

In the control group № 1, the death of small mollusks (*L. truncatula*) was noted at 100%, and swamp mollusks (*L. palustris*) – at 99.7%. Motor activity of invertebrates, swelling of the leg epithelium of mollusks, and abundant mucus release was also observed during microscopy. In the control group № 2, minor gastropod death was noted in the *L. truncatula*, probably from the effects of the parthenitic stages of parasite *F. hepatica* development. The death of the *L. palustris* in control № 2 was not noted. The release of mucus and the manifestation of the motor activity of mollusks in the experiment were not noted.

In the field, the alcohol extract of *S. Latifolia* was tested on mollusks of 5 species (*L. truncatula*, *L. palustris*, *P. planorbis*, *P. corneus*, *P. fontinalis*). 98.1-100% death of gastropods was observed in 4 biotopes (Table 2) 8 hours after exposure to a molluscicidal remedy. Within 15 minutes, mollusks showed motor activity and mucus release. With gastropod microscopy (increase x2 - 20) in 100% cases, edema of the leg cover epithelium and abundant release of clear mucus (protective reaction of the invertebrate organism to the molluscicidal remedy of contact action) were noted. After a week, empty shells were recorded in biotopes 1-2.

The working 1% aqueous solution of *S. Officinalis* extract showed an efficiency of 98.3% - 100% (control № 1). Copper sulfate showed 100% molluscicidal efficiency (control № 2). In biotope, № 4, after 7 days of observations, two dead lake frogs (*P. ridibundus*) and one horseleech (*H. sanguisuga*) were found.

In the fifth biotope, the motor activity of gastropod mollusks and their death were not noted (control № 3). By the 7th day of observations in the biotope, an increase in clutches of mollusks caviar was noted.

In field tests, the use of *S. Latifolia* extract did not show

the death of amphibians and other invertebrates. In the laboratory and the field, plant burns (wild cereal plants, plantain *Alismataceae*, clover) and algae covering the soil with mollusks were not noted.

When studying LD₅₀ and LD₁₀₀ toxic properties of vegetable remedies are established, that double intragastric and subcutaneous administration of the working solution in the maximum possible doses did not cause toxic effects on the body of laboratory animals. According to the classification of toxic substances GOST, 12.1.007-76 [19] molluscicidal remedy belongs to class IV "low-risk substances". As a result of studying cumulative properties at K_{cum} made oral administration to rats was more than 3.0, that is exceeded the value of ≤ 1. According to GOST [17] and toxicological reference book [16] for medicinal products, this drug does not have cumulative properties. Toxicological statistics data is not shown.

Discussion

Copper sulfate (copper cupros) is the most commonly used molluscicide. It is considered the standard of molluscicidal activity [13]. In order to ensure the safety and efficiency of molluscicide processing, the development and application of directed preparations based on plant raw materials are most effective [19,20]. They are characterized by ecological purity and directivity of action (they are less toxic to other communities of biocenosis organisms of the selected biotope) [12,13,19-22].

When developing the next molluscicide preparation from a huge list of the species of the plant kingdom, special attention should be paid to those that contain surfactants (saponins). These active ingredients have is high stabilizing activity and good solubility in the water. Plant raw materials, which have triterpene saponins in chemical composition, can be a promising and effective means of combating land mollusks and pasture helminths of the animals and birds.

The retrieved data are consistent with the results of VV Gorokhov and VS Osetrova [13], who tested a prototype of a plant remedy based on *S. Officinalis*. The active substance in the infusion from *S. Officinalis* having molluscicidal activity was saponins - surfactants. Gorchakov VV (2007, 2016, 2017) [20-22], Gorchakov VV, et al. [19] developed a technology for preparing preparations (powder, water, alcohol, ammonia extracts) based on the biomass *Bergenia crassifolia*, *Picea abies*, *Quercus robur*. Due to preventive processing with plant molluscicides against intermediate hosts, it is possible to reduce infection with trematodes of animals by 10 - 14 times.

Since the extract used is made from plant raw materials (roots, stems, flowers of *S. Latifolia*), there will probably be no tangible environmental consequences.

Conclusion

The results of experiments conducted in the pastures



indicate a high molluscicidal activity of the studied plant remedy on freshwater mollusks, intermediate hosts of *F. hepatica*. Its efficiency of mollusk during processing (10.0 g/l) is from 98.1% to 100%. Previously, it was found that the remedy has a molluscicidal effect against mollusks of the family *Lymnaeidae*. The results obtained indicate the possibility of using the extract from the plant *S. latifolia* as a molluscicide for the health improvement of pastures and cattle when carrying out a complex of therapeutic and preventive measures against fasciolosis and other trematodes of ruminants.

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Ethical standards

This experimental study was conducted by the guidelines for the protection of the health of test animals and the conduct of experiments on natural biotopes, which was approved by the committee on the work of laboratory animals and environmental protection (Protocol of Invasion Diseases Section №1. 11/02/2019; European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. ETS No.123. Strasbourg, 18/03/1986) [22].

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