Plant molluscocide for prevention of animal helminthosis

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Abstract. Soft-bodied are intermediate hosts of helminthias, in the body of which a number of 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 aim of the work was to determine the molluscicidal effectiveness of a plant remedy based on Saponaria officinalis in conditions of natural pastures. The material for work was the green mass of the plant Saponaria officinalis 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. Field work in natural pastures was carried out on 6 biotopes with an area of 5-23 m². Three species of gastropods were recorded from freshwater mollusks in the biotopes: Physella acuta, Lymnaea truncatula and Lymnaea 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 trematodosis pathogens. The effectiveness of the developed molluscocide in relation to gastropods when treated with a working solution (10.0 g/l) is from 92.3 to 100%.

1. Introduction

Helminthic diseases of domestic animals and birds cause significant economic damage to agriculture and forestry [1, 2]. Pathogens of helminthiasis, especially pasture ones, mature in their life cycle in intermediate hosts-insects, crustaceans, reptiles, as well as in mollusks. Gastropods are the sources of most trematode and nematode infestations of farm animals. In their body, a number of stages of development of larva forms of helminthiasis occur [3].

Additional hosts Fasciola hepatica are demanding comfortable living conditions. In temporary puddles, hollows and ponds with a sandy bottom limneids meet focal. Mollusca do not like peatlands, so they can’t be found in them. Their biotopes mostly function in reservoirs with clay and silt bottoms with a pH of 6.6-7.2 units. In places where ruminants graze, their settlement density is the highest. Usually, due to the insolation of the bottom of the biotope at a water depth of 3-10 cm, the optimal temperature of the habitat is 21.0-25.5°C.
Mollusca are resistant to physical, chemical and biological factors due to the thickness of the scintillating layer of the integumentary epithelium and the density of the integumentary collagen fibers that form the skin and muscle invertebrate bag. Preventive treatment of pasture meadows against mollusk is usually carried out during the period of activation of mollusk (the period of spawning, rapid development and growth of the young generation) at a water temperature of not less than 13°C and a pH of not higher than 7.6. Methods of application molluscocidal remedies are elected, depending on the biological characteristics of invertebrates, their ecology, habitats, and other factors. Synthetic drugs (compounds of copper, arsenic, zinc, barium, mercury, polychloroterpenes, alcohols, phenols) are promising, but their use in large areas is quite expensive.

Plant remedies are eco-friendly and slightly toxic relative to mammals, fish, amphibians, hydrobionts and vegetation. This group of molluscocides is inexpensive and available for use.

The synthesis of cheap and effective remedies with a molluscocidal effect is an urgent task as a control and prevention of parasitic diseases of farm animals of walking (pasture) content.

2. Research purpose
The purpose of this work was to determine the molluscocidal effectiveness of a plant remedy based on Saponaria officinalis against freshwater gastropod molluscs in laboratory conditions and natural pastures.

Folk medicine uses the Saponaria officinalis picking as a strong expectorant and antitussive agent used to thin thick bronchial mucus for bronchitis, pneumonia, whooping cough, painful cough. Medicinal products based on it show antimicrobial, diuretic, choleric, anti-inflammatory, wound healing, anti-rheumatic and antitumor effects [5-10].

3. Materials and methods of research
Saponaria officinalis plants were extracted entirely from the natural areas of the Central Region of Russia. Picking and drying of green biomass was carried out in the period from July to October of 2018-2019 to achieve the maximum yield of vegetation mass of the plant. At this time there is the flowering, fruiting and most importantly, the accumulation of surface-active substances (saponins). Precisely, these substances have a disastrous effect on the life of molluscs. Saponaria officinalis was dug up with roots, washed in tap water, dried in the shade without access to sunlight until the humidity in the biomass reached 15-18%.

The mollusocide powder from the roots, leaves, stems, flowers and seeds of the plant was prepared by grinding the dried picking in a mortar with a pestle to the size of 1-3 mm particles. Next, the Saponaria officinalis powder was extracted with ethyl alcohol in a mass ratio of 1:100 for 24 hours at a constant temperature of 28±2°C and stirring at 100±1 rpm. The resulting extract was filtered through a filter paper. The filtrate was concentrated in a Heigolph rotary evaporator at pressure of 4±2 kPa, temperature of 45±2°C and 280 rpm, followed by drying the concentrate for a day in a vacuum desiccator at a pressure of 10 kPa. The resulting product (concentrate) was an amorphous gel-like mass of dark green color with a specific gentle smell and well soluble in water. Then, the concentrate was dissolved to the desired concentration and received molluscoidea remedy [11].

The resulting new form of Saponaria officinalis was introduced into the biotopes of mollusk habitat in the form of a working water solution using a sprayer (spray). Preliminary studies were conducted in the laboratory for monitoring and forecasting parasitic diseases of animals on models of artificial biotopes [12]. Populations of mollusks were selected from natural biotopes of the Moscow Oblast of Central Russia. As objects for research, we used 2 types of pond fish: small (Lymnaea truncatula) and marsh (Lymnaea palustris). These gastropods are intermediate hosts of the causative agent of fascioliasis by Fasciola hepatica.

Molluscs were kept in crystallizers with a volume of 3-5 liters for 12-30 pieces, plastic cuvettes with a volume of 30-50 liters for 180-250 pieces, aquariums with a volume of 60-80 liters for 270-300 pieces with the clay, settled swamp water, field and aquatic plants. For additional lighting of biotopes,
phytolamps of the Fluora type were used, and aquarium compressors were used for aeration of soil and water. The ambient temperature in malacaria was 20 ± 2 °C, the pH of the soil on which the imported mollusks, egg laying and juvenile soft-bodied 6.6-6.8 units lived. The viability of molluscs was detected by the Levenhuk 3ST binocular microscope when they were heated on the Morozov table for 5-10 minutes. During the registration of motor activity for a period of 5-15 minutes, gastropods were considered viable. Studies were conducted 3 months after the removal of components from natural conditions. As a control of the molluscostatic effect of the new form of the drug, a 0.1% decoction of Saponaria officinalis was used [13]. The results of studies and observations were taken into account within 30 days after exposure to molluscocide in the laboratory.

Field work in natural pastures was carried out on the territory of the Moscow Oblast of the Central Region of Russia in 2019. Processing of 6 biotopes was carried out on an area of 5.23 m². 3 species of gastropods were recorded from freshwater mollusks in natural habitats: Physella acuta, small (Lymnaea truncatula) and marsh (Lymnaea palustris) pond fish. The species identity of mollusks was determined by the Glauer determinant [14]. In biotopes, as a control, disinvasion was carried out with copper vitriol (2 g per 1 m² of the biotope or 0.2 g/l of the working solution with an exposure of 1 h, according to the Instruction on measures to prevent and eliminate animal diseases with helminthiasis). Gastropods on pastures were processed during the active state of mollusks: at the end of May (after the meltwater subsidence) and in August (on the next day after precipitation, in cloudy wet weather, in the morning during strong dew at a water temperature of not less than 14°C). Organic and mineral fertilizers were not applied during the experiments, and animals were not grazed in these areas. The viability of gastropods was determined the next day and 5 days after treatment with remedies. Also, the biotope was inspected for the presence of other dead organisms (insects, amphibians, etc.).

The study of toxic LD50, LD100 (in white mice and rats of both sexes) and cumulative Kcum (in white mongrel male rats) properties of the molluscocide from Saponaria officinalis was performed using standard methods [15].

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".

4. Results and discussion
Working remedies were dispersed with a 1000 ml manual Grida sprayer at the rate of 100 ml per 1 m² of the artificial biotope area. Preliminary studies conducted in the laboratory have shown 100% effectiveness of the alcohol extract of Saponaria officinalis (table 1). Molluscocide remedy was effective at 1% strength dilution in tap water. During the first 10-15 minutes, gastropods showed high motor activity, and pond fish 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, the death of small pond fish was noted by 6.2%, and swamp fish by 2.5%. Mucus and motor activity were not detected in freshwater gastropods. Apparently, the concentration of saponins in the working solutions of the molluscocide agent was significantly lower compared to the active substances from the alcohol extract.

| Mollusc species | Number of processed molluscs | Number of dead molluscs | Dead molluscs (%) |
|-----------------|------------------------------|-------------------------|-------------------|
| L. truncatula   | 267                          | 267                     | 100               |
| L. palustris    | 181                          | 181                     | 100               |
| Control group (0.1% decoction Saponaria officinalis) | | | |
| L. truncatula   | 257                          | 16                      | 6.2               |
| L. palustris    | 204                          | 5                       | 2.5               |
In the field, the alcohol extract of *Saponaria officinalis* was tested on mollusks of 3 species (*L. truncatula*, *L. palustris* and *P. acuta*). 100% death of gastropods was observed in 3 biotopes (table 2). The lethal effect of the test agent in relation to gastropods was noted among the small pond fish and the pointed fish. From 92.3 to 99.1% of deaths were recorded among small and swamp pond fish.

In the control group, 100% soft-bodied death was observed in both biotopes. After 1 and 5 days, empty shells of gastropods *P. acuta*, *L. truncatula* and *L. palustris* were observed in the treated territories of biotopes.

**Table 2.** Molluscocidal efficiency of the 1% working solution of a drug based on *Saponaria officinalis* under natural conditions.

| Biotope number | Biotope area (m²) | Soil and water temperature (°C) | Mollusc species | Number of detected mollusc | Number of shells collected | Dead mollusc (%) |
|----------------|-------------------|---------------------------------|----------------|---------------------------|---------------------------|-----------------|
| 1              | 5                 | 17.2                            | *L. truncatula* | 19                        | 19                        | 100             |
| 2              | 8                 | 23.1                            | *P. acuta*,     | 8                         | 8                         | 100             |
|                |                   |                                 | *L. truncatula* | 13                        | 12                        | 92.3            |
| 3              | 23                | 20.6                            | *P. acuta*,     | 3                         | 3                         | 100             |
|                |                   |                                 | *L. truncatula* | 31                        | 30                        | 96.8            |
| 4              | 18                | 22.4                            | *L. palustris*  | 318                       | 315                       | 99.1            |
| 5              | 12                | 17.0                            | *P. acuta*,     | 11                        | 11                        | 100             |
|                |                   |                                 | *L. truncatula* | 115                       | 115                       | 100             |
| 6              | 8                 | 22.5                            | *L. palustris*  | 328                       | 328                       | 100             |

1 hour after exposure to the test agent in the concentration of the 1% aqueous solution, there was a slight death of crustaceans (Daphnia 5-11% and Cyclops 7-15%). After 18-20 days, their number in the biotopes was restored. Animals amphibians (frogs, newts) have gone 1-3 on the day. The effect of the *Saponaria officinalis* based products on plant flora was not observed.

In the control biotopes, the death of crustaceans was marked significantly (Daphnia 65-100%, Cyclops 70-100%), after 30 days, the recovery of their populations was not noted. In addition, the body of a grass frog (*Rana temporaria*) was found on biotope №2 on day 5.

When studying the lethal effect of a molluscocidal agent, it was found that twice intragastric and subcutaneous administration of a drug based on *Saponaria officinalis* in the maximum possible doses did not cause a toxic effect on the body of warm-blooded animals, which allows us to conclude that it is harmless. According to the GOST standard 12.1.007-76, the remedy was assigned to the IV hazard class "low-hazard substances" [15].

The presented data are consistent with the results of V. V. Gorokhov and V. S. Osetrova [13], who tested a prototype of a plant remedy based on *Saponaria officinalis*. Also, research by V. V. Gorchakov [16, 17] shows that many other plant remedies (*Quercus robur*, *Polygonum bistorta*) have a molluscocidal effect. Due to preventive treatment with plant molluscicides against intermediate hosts, it is possible to reduce infection with fascioles and other trematodes of animals by 10-14 times.

5. **Conclusion**

The results of experiments conducted in the pastures indicate a high molluscocidal activity of the studied plant agent on pond fish, intermediate hosts of fascioles pathogens. Its efficiency in relation to mollusk during processing (10.0 g/l) is from 92.3 to 100%. Previously, it was found that the remedy has a molluscocidal effect against mollusks of the families *Lymnaeidae* and *Planorbidae* [11]. The results obtained indicate the possibility of using an extract from the plant *Saponaria officinalis* as a
molluscocide for the sanitation of pastures and cattle runs during the complex of therapeutic and preventive measures for fascioles and other trematodoses of ruminants.

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