

Ecologically Safe Composition to Protect Animals from Parasitic Mites

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The publication discusses the results of using a composition based on pine resin against parasitic animal mites. Both the toxicity and repellency of the developed composition against parasitic mites, as well as the possibility of using the resulting preparation, based on turpentine oil, as an alternative to chemical acaricidal compounds, are considered. The effectiveness of the developed composition was studied both in laboratory and in real conditions. When contaminating, under laboratory conditions, hungry individuals, their 100% death was observed after 96 hours. In real conditions, the number of dead mites that fell from the skin after treatment of animals was recorded. It has been established that hungry adult ticks die completely within 3-4 days, and well-fed individuals - after 4-5 days. The clinical condition of the cattle in the experiment was within the normal range, milk yields did not decrease. Parasitic mites do not settle on the body of an animal for 25-28 days when the skin of animals is treated with a working solution of a composite preparation. Imported acaricides on the market protect animals from attack by parasitic mites for only 12-15 days, which is probably due to the repellent properties of turpentine oil and prolonged acaricidal action of the composite preparation. Clinical, hematological, biochemical, as well as macro- and micromorphological studies (3 hours after applying the composite preparation to the skin of experimental rabbit animals) showed a slight decrease in the activity of blood cholinesterase, hemoglobin, as well as the number of erythrocytes and leukocytes. 72 hours after contamination, all indicators are restored to their original level, without signs of intoxication. 1 hour after the treatment of rabbits with a composite preparation according to the "rubbing into the skin" principle, moderate capillary hyperemia and perivascular infiltration were observed on the skin and regional lymph nodes. 6 hours after rubbing the drug into the skin, these changes are no longer observed, which indicates that these changes are not the result of the toxicity of the drug or its accumulation in the skin. Consequently, composite drug prepared on the basis of gum turpentine fully complies with modern requirements for veterinary preparations.
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acaricidal product, composition, parasitic ticks

The greatest harm to farm animals is caused by ticks of the Ixodidae family, which are predominantly common in Georgia [1]. The likelihood of animals getting sick is 40-50% if measures are not taken to protect them from the attack of parasitic mites. In case of delay with expensive treatment of infected animals, the mortality rate is 80-90%.

At present, insectoacaricidal preparations prepared on the basis of synthetic pyrethroids are widely used against parasitic mites [2-5]. Unfortunately, their use is accompanied by a number of undesirable phenomena (environmental pollution, harm to human health, adverse effects on beneficial insects, etc.).

Taking into account the requirements of the European Union and the World Health Organization (Communique of the European Commission 640 – European Green Deal, Brussels, December 11, 2019), studies have been carried out aimed at obtaining "botanical insecticides" (as an alternative to synthetic insecticides) based on natural compounds, which is topical in recent years [6, 7].

Composite insecoacaricide against parasitic mites of animals, safe for humans and animals, obtained on the basis of gum turpentine (essential oil isolated from the resin of coniferous plants). It has been established that the content of gum turpentine gives the compositions repellent properties (the specific and persistent smell of gum turpentine suppresses the smell of the target product of insects and causes their disorientation) and a prolonged insect-acaricidal effect, which is confirmed by the results of testing these compositions against plant pests [8-11].

In order to increase the effectiveness of the target drug, a small amount (no more than 2%) of a synthetic pyrethroid – cypermethrin was introduced into the composition of the composition. Cypermethrin, unlike other pyrethroids, has low volatility, low mobility, and rapid decomposition (according to the results of many years of use of pyrethroids). Its toxicity is moderate, irritating to eyes and skin. It does not have a teratogenic, mutagenic or

carcinogenic effect at doses up to 140 mg/kg. Therefore, small doses are safe for humans and the environment [12].

Aggregates Water-turpentine oil microemulsion formed in the working solutions of the developed composition lead to a gradual release of synthetic pyrethroid molecules from the system and determine the prolonged insecticidal effect of the composition [13]. As a result, due to the prolonged acaricidal action, a small amount of pyrethroid in the developed composition will be enough to cover the duration of the pest development cycle.

With temporary or permanent parasitism on the body of an animal, ticks (blood-sucking) introduce saliva into the body of the host animal, which has an anticoagulant effect, causing both general and local toxic effects. Added to this is the fact that in the process of crawling over the body of an animal, ticks irritate skin receptors, causing severe itching. Due to constant scratching, the skin is damaged, the protective function of the skin is disrupted and the best conditions are created for the penetration of microorganisms into the body from the damaged areas. Consequently, there is a high probability of developing various pathological processes, including the appearance of abscesses, the detection of dermatitis, bruising, hemorrhage, etc. [14].

Materials and Methods

Parasitic mites were collected both from the skins of animals and from livestock stalls, as well as in areas and pastures adjacent to the stalls, to determine the acaricidal effectiveness of the developed composite preparation against animal ectoparasites. Morphological studies for the identification of ticks were carried out using tables of Galuzo and Zakhvatkin [3] and an MVS-9 microscope.

The acaricidal activity of the developed composition, under laboratory conditions, was studied by direct contact (contamination) of the composition with parasitic mites. For this, 15-15 units of parasitic mites of the same species (hungry and

well-fed nymphs and adults) were placed in Petri dishes (main and control). On the body of parasites placed in the main Petri dishes, a solution of the test composition of various concentrations was applied for one minute. Parasites placed in control Petri dishes were contaminated with an inert solution (water). Three parallel experiments were carried out. Dead and paralyzed ticks were recorded after 12, 48, 72, 96, 120 and 144 hours.

The effectiveness and safety of the use of the developed composition for the treatment of the skin of domestic and farm animals was established by the effect of the composition on experimental animals in laboratory conditions according to the "rubbing into the skin" principle. After a clinical examination (pulse, respiration, activity), laboratory animals (rabbits) were divided into two groups.

In experimental animals of the first group, before treatment of their skin with the developed composition, as well as 1, 3, 5 hours and 2, 3, 7, 15 days after treatment, blood was taken from the ear vein for biochemical and hematological studies. The quantitative content of hemoglobin, erythrocytes and leukocytes in dynamics was studied by conventional methods. Rabbit skin was treated by applying an emulsion (3-5 ml per individual) to shaved skin (area 4×5 cm) containing 0.1-0.15-0.2-0.25% of the developed composition.

Within 3 days after the application of the developed composition, constant monitoring of the reaction of laboratory animals and their clinical indicators was carried out. In parallel, pathomorphological changes in dynamics were studied in rabbits of the second group. In particular, 1-6 and 24 hours after the above exposure to the developed composition, the animals were slaughtered and the material was taken for pathomorphological examination. The material was taken from the skin and subcutaneous tissue (from the site of exposure to the developed composition), liver, lungs, heart, kidneys and from lymph nodes near the site of exposure to the developed composition.

Primary fixation of pathogenic material was carried out in a 10% solution of neutral formalin, then it was transferred to a 15% solution. The material was formed in paraffin, and to study pathological changes in organs and tissues, the sections were stained with a solution of hematoxylin-eosin. If necessary, the Sudan 3 staining method was used to study fatty degeneration and lipids.

The acaricidal activity of the developed composition under real conditions was studied in 4 farms (Dmanisi municipality, Georgia). In each farm, 4-5 animals (adults and young animals) were selected, the skin of which was treated with 0.0075-0.001-0.015-0.02% working solutions of the developed composition. The effectiveness of the composition was determined by the state of mites during daily observation and registration of their death and fall from the surface of the skin of the animal.

Due to the fact that the developed composition contains a small amount of pyrethroid (no more than 2% cypermethrin), studies were carried out to determine the content of the residual amount of cypermethrin in the organs and tissues of laboratory animals (rabbits) after their treatment with the developed composition.

The quantitative content of cypermethrin in analytical samples (extracts of organs and tissues of laboratory animals) was determined on a firm "Varian" model CP-3800 gas chromatograph with an electron capture detector (ECD). A 25 m x 0.25 mm CP Sil 19 CB column was used. The analysis was performed according to the Method of M.M. 268-1.3-5000.

Results and Discussion

According to laboratory studies, 100% death of parasitic mites occurs 72 hours after their contamination (0.015-0.02% solution of the developed composition), and when hungry adults contaminate with 0.0001-0.0075% solution, lethality is 78.9-95, 9%, which occurs after 96 hours.

Hematological and biochemical studies showed that 3 hours after treatment of the skin of an experimental animal (rabbit) with the developed composition, there was a slight decrease in hemoglobin in the blood. The number of erythrocytes and leukocytes also slightly decreased. 72 hours after treatment of the skin of experimental animals, these indicators are restored to their original value, without signs of intoxication (Table).

Conducted micromorphological studies found that after 1 hour in the places of treatment with the developed composition, the histological structures

of the skin are preserved. The blood vessels of the skin, mainly in the reticular layers, are dilated and filled with blood. This vascular reaction was primarily associated with the capillaries, around which, in a separate case, there was a weak serous fluid and a single leukocyte (eosinophilic) infiltration, occupying a small area. The walls of the vessels retained the histological structure. The sebaceous glands and excretory ducts are well defined. The connective tissue bag of fur is completely preserved. The nearest (regional) lymph nodes adjacent to the site of application of the composite

Table. Hematological and biochemical blood parameters of experimental animals (rabbits) before and after treatment of their skin with a developed composition

Concentration of reparation, %	Before processing with preparate	Period after processing with preparate						
		1 hour	3 hours	5 hours	48 hours	72 hours	168 hours	360 hours
Content of protein in blood serum g/dl								
0.1	7.3	7.1	7.1	7.2	7.3	7.2	7.3	7.3
0.15	7.3	7.2	7.2	7.3	7.4	7.1	7.5	7.4
0.2	6.8	6.7	6.3	7.4	7.3	7.4	7.2	7.3
0.25	7.4	7.2	7.3	7.2	7.3	7.4	7.1	7.3
Content of sugar in blood, mg/dl.								
0.1	65.0	64.0	65.1	64.9	65.3	64.7	64.3	65.1
0.15	64.3	65.2	65.2	65.0	65.2	65.2	64.8	65.1
0.2	65.1	65.1	65.0	65.2	64.9	65.1	64.4	64.8
0.25	65.0	65.1	65.0	64.8	64.9	65.1	65.0	65.0
Content residual nitrogen in blood, mmol/l.								
0.1	22.0	21.0	22.0	22.0	24.0	20.0	22.0	21.0
0.15	24.0	23.0	22.0	24.0	23.0	23.0	24.0	24.0
0.2	21.0	20.0	21.0	22.0	24.0	22.0	23.0	22.0
0.25	23.0	23.0	22.0	23.0	21.0	20.0	21.0	23.0
Content of bilirubin in blood, mg/dl.								
0.1	0.18	0.18	0.20	0.21	0.19	0.18	0.20	0.20
0.15	0.22	0.19	0.21	0.21	0.20	0.20	0.18	0.19
0.2	0.21	0.22	0.21	0.22	0.18	0.20	0.21	0.22
0.25	0.21	0.21	0.20	0.21	0.22	0.22	0.23	0.22
Content of hemoglobin in blood, g/dl								
0.1	11.4	11.3	11.4	11.3	11.1	11.2	11.3	11.5
0.15	11.4	11.4	11.4	11.3	11.0	11.1	11.3	11.6
0.2	12.0	11.6	11.2	11.2	10.8	11.1	11.4	11.4
0.25	12.1	11.3	11.2	11.0	11.6	11.0	11.2	11.5
Content of leukocytes in blood, $10^3/\mu\text{l}$								
0.1	8.1	8.0	8.0	7.9	7.9	8.2	8.4	8.4
0.15	8.4	8.4	8.2	7.9	7.8	8.0	8.3	8.3
0.2	8.3	8.2	8.0	7.8	7.8	8.1	8.1	8.5
0.25	8.8	8.7	8.6	8.0	7.8	8.0	8.2	8.7
Content of erythrocytes in blood, $10^6/\mu\text{l}$								
0.1	5.1	5.1	4.9	4.7	4.9	5.0	5.2	6.1
0.15	5.7	5.7	4.6	4.8	4.8	4.6	5.1	5.0
0.2	4.9	5.0	4.3	3.9	3.9	4.3	5.0	5.3
0.25	6.1	6.0	4.5	4.1	3.7	4.8	5.2	5.4

preparation (with a characteristic grayish-white color and consistency) are not enlarged in volume.

According to the histological examination, the structure and histological structure of the lymph node are preserved, with clearly defined primary and secondary follicles (light zones remain in the secondary follicles). The follicles are overflowing with lymphocytes and reticular cells. Hyperemia persists in the node capsule and medulla, expressed mainly in the venous capillaries. The anatomical parameters of the liver are preserved, no macromorphological changes are observed. Pathological anatomical studies have shown that in the venous capillaries there is a mild vascular reaction (in the form of hyperemia). Micro- and macromorphological changes in the heart, lungs and kidneys were within the normal range and no deviations from them were observed.

Hyperemia and weakness of vascular infiltration, previously expressed in the skin of the animal, were no longer observed 6 hours after the application of the developed composition to the body of the animal. The clinical signs of the animals were within the normal range. Mild hyperemia in the regional lymph nodes returned to normal. Deviations from the norm of histological changes in the studied organs were not registered. A similar picture is expressed 24 hours after application of the developed composition to the body of a laboratory animal.

The content of cypermethrin in the organs and tissues of experimental animals (rabbits) was not found. An exception is the skin of an animal with hair, where the content of cypermethrin was 0.004 ± 0.001 mg/kg, which can be considered as a residual amount of the developed composition.

The study of the effectiveness of the developed composition in real conditions (in 4 households, where 20-25 ticks were found on the body of each animal) showed that after treating animals with working solutions (of developed composition),

hungry adults die within 3-4 days, and well-feds – in 4-5 days. The clinical condition of the animals (in the experiment) was within the norm. The decrease in milk yield was not recorded.

According to the results of visual observation of animals on the skin of cattle treated with solutions of the developed composition, the presence of mites was not detected within 20-25 days. On animals from the same herd, with untreated skin, an average of 10-15 individuals were found.

Conclusión

On the basis of the conducted studies, it was found that the composition prepared on the basis of gum turpentine is effective against parasitic mites of animals and has a prolonged acaricidal effect. During the study, no signs of the toxic effect of the developed composition on experimental animals were observed. Therefore, it can be considered safe for humans, animals and the environment.

Deviation from the norm of some vital signs (in the first 6 hours after skin treatment with the developed composition) of the organism of an experimental animal is not a consequence of the toxicity of the composition, or its accumulation in the skin. Most likely, this should be caused by manipulations carried out on experimental rabbits, as a result of a stress reaction that does not go beyond the effects on the body.

The developed composition fully complies with the requirements for veterinary drugs: easy to prepare and easy to use; Safe for human and animal health; Do not have cumulative properties; It is characterized by a high spectrum of action and the effect of prolonged acaricidal action.

The developed composition prepared on the basis of gum turpentine is an innovation, its large-scale use will greatly contribute to the improvement of the maintenance of farm animals and the production of safe livestock products.

ბიოტექნოლოგია

ეკოლოგიურად უსაფრთხო აკარიციდული საშუალება ცხოველთა დასაცავად პარაზიტული ტკიპებისაგან

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(წარმოდგენილია აკადემიის წევრის ვ. ციციშვილის მიერ)

ნაშრომში წარმოდგენილია ფიჭვის ფსიდან გამოყოფილი ტერპენტინის ზეთის საფუძველზე მომზადებული კომპოზიციის ცხოველთა პარაზიტული ტკიპების წინააღმდეგ გამოყენების შედეგები. განხილულია პარაზიტული ტკიპების მიმართ, ამ მცენარეული ნაერთის როგორც ტოქსიკური მოქმედება და დამაფრთხილებელი თვისებები, ისე დღეისათვის ცხოველების პარაზიტული ტკიპებისგან დასაცავად გამოყენებული აკარიციდული ნაერთების შესაძლო ალტერნატივა. შესწავლილია შემუშავებული კომპოზიციის ეფექტურობა ლაბორატორიულ პირობებში და რეალურ გარემოში. შემუშავებული კომპოზიციით, მშეიერი იმაგოების ლაბორატორიულ პირობებში დასნებოვნებიდან 96 საათის შემდეგ, დაფიქსირდა მათი 100% -იანი სიკვდილიანობა. ხოლო რეალურ გარემოში, სადაც ტკიპების სიცოცხლისუნარიანობა ფიქსირდებოდა კანის საფარველიდან ჩამოცვენილი, დაღუპული ტკიპების რაოდენობით. ტკიპების მშეიერი იმაგო ფორმები მთლიანად იხოცება 3-4 დღის განმავლობაში, ხოლო მამდარი ინდივიდები – 4-5 დღის შემდეგ. ცდაში მყოფი პირუტყვის კლინიკური მდგომარეობა იყო ნორმის ფარგლებში. კომპოზიციური პრეპარატის სამუშაო ხსნარებით დამუშავებულ ცხოველის კანის საფარველზე პარაზიტული ტკიპები აღარ სახლდებიან 25-28 დღის განმავლობაში. ბაზარზე არსებული იმპორტული აკარიციდული პრეპარატები კი ცხოველებს პარაზიტული ტკიპების თავდასხმისაგან იცავს მხოლოდ 12-15 დღის განმავლობაში. ეს გარემოება, სავარაუდოდ, განპირობებულია ტკიპების მიმართ ტერპენტინის ზეთის დამაფრთხილებელი თვისებებით და კომპოზიციური პრეპარატის გახანგრძლივებული (პროლონგირებული) აკარიციდული მოქმედებით. ჩატარებული ფიზიკურ-ქიმიური, კლინიკური, ჰემატოლოგიური, ბიოქიმიური, ასევე მაკრო და მიკრომორფოლოგიური გამოკვლევებით დადგინდა, რომ პირეტროიდის (ციპერმეტრინი) შემცველობა ფიქსირდება მხოლოდ საცდელი ცხოველის (ბოცვერი) თმის კანის საფარველში არაუმეტეს 0,001 მგ/კგ ოდენობით. ბოცვერების კანზე კომპოზიციური პრეპარატით დასნებოვნებიდან 3 საათის შემდეგ შეინიშნება საცდელი ცხოველის სისხლში ჰემოგ-

ლობინის უმნიშვნელო შემცირება. ასევე უმნიშვნელოდ მცირდება ერთოროციტების და ლეიკოციტების რაოდენობა. პრეპარატი დასნებოვნებიდან 72 საათის შემდეგ, ხდება ყველა მაჩვენებლის საწყის დონემდე აღდგენა, ინტოქსიკაციის რაიმე ნიშნით გამოვლენის გარეშე. კომპოზიციური პრეპარატის ბოცვრების კანში შეზღვევიდან 1 სთ-ის შემდეგ, კანსა და რეგიონალურ ლიმფურ კვანძებში დაფიქსირებულია სუსტად გამოხატული კაპილარული ჰიპერემია და პერივასკულარული ინფილტრაცია. პრეპარატის შეზღვევიდან 6 სთ-ის შემდეგ ნორმიდან გადახრა აღარ აღინიშნება, რაც იმის მაჩვენებელია, რომ აღნიშნული ცვლილება არ არის პრეპარატის ტოქსიკურობის ან მისი კანში კუმულაციის შედეგი. ტერპენტინის ზეთის საფუძველზე მომზადებული კომპოზიციური პრეპარატი სრულად აკმაყოფილებს ვეტერინარულ პრეპარატებისადმი წაყენებულ თანამედროვე მოთხოვნებს, შესაბამისად, პრეპარატის ფართომასშტაბიანი გამოყენება მნიშვნელოვნად შეუწყობს ხელს საქართველოში პირუტყვის მოვლა-პატრონობის გაუმჯობესებას და მეცხოველეობის პროდუქციის წარმოების გაზრდას.

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