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Development of a new insecticidal and acaricidal preparation for protection of cattle from zoophilous flies in Northern Kazakhstan

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Abstract
In the Qostanay University, a new veterinary preparation was developed based on permethrin within the framework of a research project on the topic "Creating a new highly insecticidal, acaricidal, and larvicidal preparation of synthetic pyrethroids to protect cattle from zoophilous flies in feedlots and pastures". Permethrin is a synthetic pyrethroid with highly insecticidal and acaricidal properties, prolonged action, the "knockdown" effect with respect to the zoophilous flies and dipterous blood-sucking insects. With account of the high efficiency of the preparation, which allows influencing on the population density of imago and immature stages of zoophilous flies, the preparation was given the original name of "Entomotsid". Thanks to proper selection of the formulation at the development of the preparation and the presence of selected components in its composition, the preparation showed high insecticidal and larvicidal effect against larvae and imago of parasitic insects. It can retain its activity at a temperature between −10°C and +37°C.

Keywords: Permethrin; synthetic pyrethroids; insecticide; Entomotsid.

Introduction

In modern industrial livestock farms in Northern Kazakhstan, in order to reduce the number of parasitic insects, they began to give preference to universal preparations of the synthetic pyrethroids group.

These preparations have a long-lasting insecticidal effect when sprayed onto the hair of animals. And when used in small quantities, they do not accumulate in organs and tissues and are not excreted with the milk of the treated animals. Taking into account the demand in the market, new combined prolonged preparative forms appear, which differ in the target efficiency, long residual effect, and high degree of safety for both specialists, who perform the treatment, and for staff [1,2].

In this regard, within the scientific project at the A. Baitursynov Qostanay State University, we created for the first time a new experimental insecticidal and acaricidal preparation based on the synthetic pyrethroid – Permethrin.

Permethrin (the chemical name – 3-phenoxybenzyl ester of 3-(2.2-dichloroethenyl)-2.2-dimethylcyclopropane carbolic acid) is applied as an insecticidal and acaricidal agent. Its effect is determined by impaired ion permeability of the sodium channels and inhibition of the processes of polarization (repolarization) of the membrane of the nerve cells of lice, fleas, mites (including itch mites), and other ectoparasites of the arthropod type, which results in a paralyzing effect. The difference from other synthetic pyrethroids resides in the prolonged effect and presence of the knockdown effect [3].

In order to prevent resistance of flies to the insecticide, rotation is recommended, i.e., timely alternation of the active substances of different chemical groups. Depending on the degree of infestation, the duration of the season, and the first signs of addiction of the population of flies, the used preparations are suggested to have the active substance changed. A correct analysis of the history of using the preparations against flies plays an important role in choosing

the right insecticide. Therefore, after prolonged use of the same active substance during past seasons, which substance is a part of a certain insecticide, the change of the preparation will play the decisive role in successful elimination of flies in farms [4].

At developing the formulation of the preparation, the focus was made on the indicators of quality, standards, and regulations. In accordance with the GOST state standards, the quality indicators of preparations are divided into indicators of purpose, reliability, manufacturability, safety, efficiency, and use of veterinary preparations.

Materials and Methods

The development of the insecticidal and acaricidal preparation, as well as the study of its physical and chemical properties, was carried out in the premises of the laboratory of the Innovative Research and Educational Center of the RSE "A. Baitursynov Qostanay State University".

Determination of the mass fraction of one active substance was carried out in the laboratory of toxicology of pesticides of the "Kazakh Scientific Research Institute of Plant Protection and Quarantine" LLC, Almaty (Kazakhstan).

Development of the preparation formulation, manufacture of the test sample, study of physicochemical properties of the preparation, mutual influence of the components on the efficiency and parameters of application and shelf life of the preparations were carried out in accordance with best practices.

The stability of the preparation during storage was determined by selecting the percentage content of constituent substances. The main criteria for the stability indicator were the absence of sediment and delamination of the preparation. The studied drug was placed in a test tube with ground stopper and kept in a thermostat at the temperature of +37°C during 30 days. After expiration of a certain period, they checked it for the presence of any aggregate state changes.

To determine the cryo resistance, the experimental sample of the preparation was placed in a test tube with a ground stopper, and then put in the freezer at −10°C for 30 days. Thereafter, the change in the aggregate state of the preparation (stratification, turbidity of the solution, crystallization of the active ingredient – a.i.) was determined.

Following the results of these experiments, they calculated the temperature range of the preparation storage. The persistence of the aqueous emulsion of the preparations was determined in accordance with the state standard GOST 16291-79 Pesticides, the method for determination of emulsion stability. The study of the repellent and insecticidal efficiency, as well as the development of regulations for use of the preparations, was carried out by the guidelines [5,6].

Results and Discussion

According to some researchers, it was found that the treatments should always cover all livestock. The previously recommended method of partial treatment of animals in a herd turned out unacceptable, since such treatment had little effect on reducing the number of imago zoophilous flies in the nature [7].

In this regard, it was initially decided during the preparation development to conduct a series of laboratory tests. The progress of the development of a new insecticidal and acaricidal preparation consisted of four stages of laboratory work:

The first stage comprised dissolving the active substance in organic solvents. We used permethrin as an active substance because it is a highly effective pyrethroid of broad-spectrum effect against all types of malicious crawling and flying arthropods (flies, horse flies, mosquitoes, fleas, mites, etc.). It has a high point of boiling (200°C) and melting (34-39°C). Xylene was used as a solvent because permethrin is poorly soluble in water at room temperature.

Second stage is the dissolution of the "synergist" of the insecticidal effect pyrethroid, wherein alcohols of the organic compounds class were used. To enhance the effect of the active substance, we used an antiparasitic preparation, because some of its components may contribute to increasing the acute insecticidal effect.

In the third stage, in order to ensure that the preparation would maintain its physical state at rapid temperature changes and would not lose its insecticidal and acaricidal, larvicidal, and repellent properties, we used an artificial polymer.

It is resistant to most organic solvents, petroleum products, acids, and alkalies, to light and microorganisms, which allows controlling

the release of active ingredients. As a conventional solvent, we used warm distilled water (22°C).

In the final fourth stage, we mixed the resulting solutions in a separate vessel, and then added flavoring fixing fluids. All was stirred thoroughly.

In the end, the 7% emulsifying concentrate of permethrin Entomotsid was developed, which was viscous white liquid with a faint specific odor.

Determination of Physico-Chemical Properties of the Entomotsid Preparation

The main criteria for the physical properties of the preparation were absence of sediment and delamination of the preparation. With this purpose, the obtained amount of the preparation was divided into three equal portions and placed in different temperature conditions. One part of the preparation was placed in an incubator ($t = +37$°C), the second one – in a cryostat ($t = -10$°C), and the third one was left at room temperature (18°C, and humidity of 60–70%).

The experimental samples of the preparation were observed for 30 days. After this period, we stated that no change in the aggregate state was detected at room temperature; there was complete freezing of the experimental sample in the cryostat; there was precipitation (with restoring original status after shaking) of the preparation in the incubator [6].

Having determined the physical properties, we proceeded to determine the mass fraction of the active substance in each experimental sample of the preparation. With this purpose, three prototypes of the Entomotsid preparation were tested in the laboratory of toxicology of pesticides of the Kazakh Scientific Research Institute of Plant Protection and Quarantine LLC, Almaty (Kazakhstan), after the restoration of its physical properties (test protocol No. 67 dated July 28, 2014). The results of the study are provided in Table 1.

The data in Table 1 show that the actual value of having kept permethrin within 30 days in the experimental sample stored in a cryostat at −10°C is equal to 0.005%, at room temperature (18°C) − 7.0%, and in an incubator at +37°C – 0.006%. Thus, the temperature range for storage of the Entomotsid preparation is not lower than −10°C and not exceeding +37°C.

Test of the Entomotsid Preparation Against Larvae and Imago of Zoophilous Flies

The experiment consisted of two phases. In the first phase of work on the selection of Musca domestica larvae and winged flies, and their delivery to the laboratory of the Innovation Research and Educational Center of the RSE "A. Baitursynov Qostanay State University", before performing the experiment, we determined the survivability of the larvae in Petri dishes without exposing to the preparation at 22-24°C and the humidity of 60-70%. A group of ten larvae specimens was observed on a daily basis. During the inspection, their motor activity was evaluated and the number of alive and dead larvae was counted (the latter were removed). These data were subsequently used as control data. When conducting experiments with the room fly, we based on the methodology of M.N. Sukhova and other researchers in a somewhat modified modification [8,9].

At the second stage, we prepared aqueous emulsions of the Entomotsid preparation in the following concentrations: 0.25%, 0.125%, 0.006% (25 ml each), water was used for the control. In order to detect the contact and residual insecticide effect in the Petri dishes on up to ten fly larvae were placed on the filter paper impregnated with aqueous emulsion of the preparation; then, the time of their death was accounted every 5 min. The observations were carried out in

Table 1: Determination of the actual content of the Entomotsid permethrin in the 7% emulsifying concentrate when exposed to different temperatures.

Seq. No.	Temperature of storage of the experimental samples of the preparation	Actual value (%)	TD for the test methods
1	−10°C	0.005	GOST 14189-81
2	+18°C	7.00	
3	+37°C	0.006	

Table 2: Larvicidal effect of Entomotsid against fly larvae at different concentrations of the preparation.

A series of experiments	Concentration (%)	The number of larvae (pcs)	Larvicidal activity in %, every					
			5 min	10 Min	15 min	20 min	25 min	30 min
5 series	0.25	10	70	90	100	—	—	—
	0.125	10	60	70	90	100	—	—
	0.006	10	20	60	70	80	90	100
	Control							
	0.25	10	65	80	100	—	—	—
	0.125	10	70	80	95	100	—	—
	0.006	10	22	66	70	75	85	100
	Control	Larval mortality was not observed						

several replicates within 24 h. Ten sets of experiments were carried out using the larvae of Musca domestica. At that time, the method of topical application of insecticides was used [10].

It should be noted that not all larvae in each group died simultaneously. Therefore, we observed the larvicidal activity of the larvae every 5 min for 1 h, which results are provided in Table 2.

Thus, the data in Table 2 show that at 0.25% concentration of the preparation, 100% death of larvae occurred in 15 min; in the second concentration (0.125%) – in 20 min, in the third (0.006%) – in 30 min.

Consequently, in all concentrations, which we used, Entomotsid has a detrimental effect on the larvae of flies, due to its contact effect. And the time of larvae death is apparently influenced by the concentration, as other conditions in the groups were similar. Furthermore, the preparation was tested against winged flies as insecticide. At that time, Entomotsid was applied to the mesonotum in the above concentrations.

The results of laboratory test of the efficiency of different concentrations of the prolonged Entomotsid preparation confirmed its contact and residual effect on fly larvae within 24 h. These data are encouraging, but they were obtained in a laboratory experiment and on a glass surface.

Conclusions

- The A. Baitursynov Qostanay State University using the developed formulation for the first time developed the 7% emulsifying concentrate Entomotsid based on synthetic pyrethroid of the permethrin.
- The invention of the Entomotsid preparation relates to veterinary parasitology, namely, to efficient means of protection of cattle at cattle-breeding sites from larvae and imago of parasitic insects. According to the results of laboratory tests, we found out that Entomotsid in 0.25% concentrations has a high insecticidal and larvicidal effect on the parasites.
- The test protocol of the Kazakh Scientific Research Institute of Plant Protection and Quarantine LLC, Almaty, No. 67 dated July 28, 2014 evidences that the Entomotsid preparation has not lost its physical and chemical properties. The optimum storage temperature was specified at −10°C to +37°C.

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