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Research Article

Effects of the *Artemisia absinthium L*. Extracts on *Eimeria tenella* Oocysts *In Vitro*

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Abstract

This study investigated the effects of wormwood extract (*A. absinthium*) on *E. tenella* oocysts *in vitro*. *E. tenella* oocysts from feces of infected chickens were isolated by flotation and divided into two groups. One part of the oocysts remained unsporulated, the other were stored in a 2.5% of potassium dichromate solution at 27°C for sporulation. The effect of 25, 50 and 100 mg/ml doses of *A. absinthium* extract on both groups of oocysts was studied for 72 hours. Baycox with 1 mg/ml dose and of potassium dichromate solution (2.5%) were used for a control. The study revealed that, regardless of the dose, *A. absinthium* extract has an anticoccidial efficacy of on sporulated and unsporulated oocysts of *E. tenella*. 100 mg/ml dose of the extract of *A. absinthium* of prevents the sporulation of 95% oocysts, and also does not preserve viability of 94% of sporulated oocysts. The same situation is when using 1 mg/ml a dose of Baycox at during incubation of oocysts at 27° C for 72 hours.

This dose of Baycox prevents the sporulation of oocysts and leads to the deformation and lysis of 96% of the sporulated oocysts. The anticoccidial efficacy of a 2.5% potassium dichromate solution for unsporulated oocysts was 3%. The results of the research revealed an anticocidial effect of the extract of bitter wormwood.

Keywords: Anticoccidial Effect; Eimeria tenella; Oocysts; Artemisia absinthium; Extracts

Unicellular protozoan parasites of the genus *Eimeria* are the causative agents of coccidiosis refers to diseases of the digestive system of birds and causes a massive defeat of the epithelial cells of the intestine, leading to diarrhea of animals, a lag in the height and weight of the birds, their mass death, leading to large economic losses in agriculture.

Coccidiosis is a dangerous source of infection for the poultry industry. It is known that each species of parasite of genus *Eimeria* in chickens have a distinctive location, immunogenicity and pathogenicity. Violation of sanitary and veterinary standards in poultry farms leads to the spread of pathogens. For involving an invasive stage into the environment, unsporulated oocysts need oxygen and temperature, humidity [7]. Under optimal conditions, sporulation occurs in 28-48 hours. During sporulation four sporocysts, each containing two sporozoites, are formed within the oocyst. *E. tenella* has high productivity and resistant to environmental factors [25].

As unsporulated oocysts are sensitive to environmental conditions, the absence of any of the factors prevents sporulation, leading to the death of the parasite.

The infection with one oocysts *E. tenella* leads to the release of 400.000 oocysts [25].

Invasion begins through the ingestion of sporulated oocysts isolated by infected birds, thus completing the endogenous stage of development, and after leaving the host organism it enters the exogenous stage. The most effective way to fight against coccidiosis in birds is to prevent the exogenous stage of oocysts. For this, all kinds of chemicals are used.

The use of chemicals that prevent sporulation of oocysts causes to environmental pollution, thereby creating environmental problems. Currently, for the prevention of the disease, the most successful solution is to prevent the process of sporulation of oocysts with the use of drugs.

Prolonged use of chemical, anticocidiostatic drugs, which are effective in fight against coccidiosis in birds, leads to the formation of resistant strains coccidiosis [1].

In this case, in order to effectively in fight against resistant strains, it is necessary to create new drugs. Since the creation of new chemical drugs and vaccines requires large funds long-term use of these new drugs leads to parasite resistance and environmental problems increased interest in the use of environmentally friendly and natural products in the fight against coccidiosis [11,12,18-21,23].

In connection with the introduction by the Council of Europe of a ban on the use of ionophore anticoccidiotic drugs and antibiotics in poultry farming for the prevention of coccidiosis, the search for alternatives is of particular relevance.

The use of plants as an alternative to anticoccidiosis drugs opens up new perspectives, especially for countries with low economic development potential. It is known that plants contain several groups of active substances, alkaloids, glycosides, tannins, saponins, flavonoids, vitamins, oils, essential acids, organic acids, and a large number of trace elements etc.

Along with the above, it is also possible the presence of such active components of the composition and mechanism of action, which in medicinal plants is still unknown. Essential oils have an inhibitory effect on the activity of pathogenic microorganisms. There are data on anticoccidioses, anthelmintic. properties of the essential oils of various plants in the literature [2,3,15,17].

In folk medicine, medicinal plants are considered harmless. Indeed, some plants, even in high doses, do not have a harmful effect on the body. Along with this, not all plants used in the treatment of various diseases are harmless. There are such plants, the improper use of which leads to serious consequences for the organisms. The purpose of the research is to assess the impact of the various doses of *Artemisia absinthium L*. extract in methanol on *E. tenella* oocysts *in vitro*.

Materials and Methods

Research had been conducted in the laboratory of biochemical bases of parasite-host relations (currently the Laboratory of Protozoology) in 2015. Fresh wormwood leaves (*A. absinthium*) after washing and drying were used in the experiment for two weeks outdoors in the shade. Dried leaves were crushed mechanically in a mortar. For the preparation of the extract from the dried leaves was used Soxhlet extractor. For this purpose, 20 g of a plant sample was extracted three times within 72 hours in 500 ml of 100% methanol. The methanolic extract after drying in a water bath, was diluted in TWEEN 80 (sorbitan monoaleate) in a ratio of 1: 4 (extract: tween 80).

Before of the experiment, the dried residue of the plant extract in methanol, filtered under vacuum, was kept at 4° C in a refrigerator. For the preparation solutions of extract at a dose of 25, 50 and 100 mg/l, were taken 2.5 mg, 5 mg and 10 mg of the dry residues of the extract separately and diluted in 100 ml of distilled water. An assessment of the anticoccidial efficacy of solutions of various concentrations of wormwood extract was given in comparison with the results obtained when using baykoks is highly effective during coccidiosis. For this purpose an aqueous solution of baykoks in a dose of 1 mg/l was used in the experiment. There has been study the effect of wormwood extract on oocysts *in vitro* isolated from chick feces and divided into 2 parts. One part of oocysts was incubated in Petri dishes in the thermostat at 27-290 C in 2.5% solution of potassium dichromate solution. During the experiment oxygen was supplied in a vessel with oocysts continuously through a pump for aquaria. During sporulation of oocysts the optimal temperature, oxygen and humidity was maintained in the thermostat. Collected sporulated oocysts were divided into 4, and unsporulated into 5 parts. It has been studied the effect of wormwood extract at 25 mg/ml, 50 mg/ml, 100 mg/ml, and 1 ml/l of Baycox on sporulated and unorulated oocysts.

Since it was established that toltrazuril show a high efficiency against the coccidia and 2.5% potassium dichromate is optimal medium for sporulation oocysts of *Eimeria*, Baycox was used as control. *Eimeria tenella* oocysts were isolated from the feces of infected chickens by centrifugation.

Each ml of suspension contained 30000 oocysts of *Eimeria tenella*.

Different concentrations of extracts of wormwood extract at 25 mg/ml, 50 mg/ml, 100 mg/ml, and 1 ml/l of Baycox located in Petri dishes were covered with filter paper with oocysts.

The experiments were carried out at room temperature 27-29^0 C for 72 hours.

Changes in the morphology of the oocysts were examined at 12, 24, 36, 48, and 72 hours by counting number of oocysts in 0.001 ml suspension per microscopic field under a 40x were recorded onto Axio Scope A1 microscope (Carl Zeis Jena). It has been assess the anticoccidiostatic activity of the solutions, by calculated oocysts after deformation and lysis, sporulated and unsporulated oocysts to the total number (%) of oocysts in comparison to data the control group. It has been calculated average data of 5 experiments by statistical processing of the results.

Results

The efficiency of disinfectants on oocysts depends on many factors including strain of oocysts and the duration of their impact on oocysts. The solutions (wormwood extract, Baykoks) used in these studies affected the oocysts of *E. tenella* for 72 hours. It was revealed that the oocysts sporulation is affected by of extract in different concentration of *A. absinthium* and Baycox on oocysts *in vitro*. Dose of 25 mg/ml extract of wormwood after 12 hours of incubation inhibits the sporulation of 89% oocysts, the effectiveness dose of 50 mg/ml and 100 mg/ml is 99.8%, 100%, respectively. With this incubation (12 hours), the efficiency of Baycox was 100%. An increase in the duration of contact of the oocysts with the extract leads to changes in their number.

Depending on the duration of the contact of the sporulated oocysts with the extract, the number of deformed oocysts increases. At dose of 25 mg/ml extract of wormwood after 24 hours of incubation the percentage of sporulation is 25%, after 72 hours of incubation -40%.

At dose of 50 mg/ml extract of wormwood after 72 hours of incubation the percentage of sporulation is 17% oocysts, and at dose of 100 mg/ml extract - 5% oocysts. It was found that 3% oocysts stored in 1 ml/l Baycox solution were sporulated, and 97% of oocysts stored in potassium dichromate solution (2,5%) at a temperature of 27°C, which is optimal for coccidia sporulation after 48 hours of incubation also were sporulated, and 3% of oocysts were unsporulated or deformed (Table 1).

Thus, it was found that an increase in the concentration of the extract solution of wormwood and the duration of contact with oocysts leads to an increase in the number of unsporulated oocysts.

It was revealed that the intensity of sporulation of oocysts decreases with increasing concentration of the solution of oocysts. A study of the effect 1 ml/l dose of Baycox revealed that the maximum sporulation occurs after 72 hours of incubation and accounts for 3% of the total number of oocysts. Research results revealed that *A. absinthiun* extract, regardless of the dose, has an anticocidial effect on unsporulated oocysts *in vitro*, and the extract at a dose of 100 mg/l has the highest effect.

After 72 hours of incubation at 27°C, Baycox (97%) used in the experiment for comparative evaluation of effectiveness has the same effect. Dose 25mg/l of *A. absinthiun* prevents sporulation 60%, dose 50 mg/l -83% oocysts (table 1).

Table 2 presents the data about the effect of wormwood and Baycox on sporulated oocysts. It has been found that plant extracts equally affect sporulated and unsporulated oocysts.

25 mq/ml dose of extract for 72 hours leads to deformation and lysis of 51%, 50 mg/ml dose -82% oocysts, 100 mg/ml dose - 94% of sporulated oocysts. The efficiency of Baycox is 96% (Tables 1 and 2).

Incubation period, hours	Different concentrations of extracts of *A. Absinthium*									$K_2Cr_2O_7$ solution			Baykoks, 1 ml/l		
	25 mg/ml			50 mg/ml			100 mg/ml								
	Sporulated oocysts%	Unsporulated, subjected to deformation and lysis, %	Efficiency, %	Sporulated oocysts%	Unsporulated, subjected to deformation and lysis, %	Efficiency, %	Sporulated oocysts%	Unsporulated, subjected to deformation and lysis, %	Efficiency, %	Sporulated oocysts%	Unsporulated oocysts, %	Efficiency, %	Sporulated oocysts%	Unsporulated, subjected to deformation and lysis, %	Efficiency, %
12	11	89	89	0.2	99.8	99.8	0	100	100	10	90	90	0	100	100
24	25	75	75	0.4	99.6	99.6	0	100	100	46	54	54	0	100	100
36	29	71	71	13.6	87.4	87.4	0	100	100	70	30	30	0	100	100
48	30	70	70	16	84	84	0	100	100	93	7	7	0	100	100
60	38	62	62	16.3	83.7	83.7	3	97	97	-	-	-	1.7	98.3	98.3
72	40	60	60	17	83	83	5	95	95	-	-	-	3	97	97

Table 1: The effect of solutions of different concentrations on the oocysts of *Eimeria tenella in vitro*.

Incubation period, hours	Different concentrations of extracts of *A. absinthium*						Baykoks, 1 mg/ml	
	25 mg/ml		50 mg/ml		100 mg/ml			
	Unsporulated, subjected to deformation and lysis, %	Efficiency, %	Unsporulated, subjected to deformation and lysis, %	Efficiency, %	Unsporulated, subjected to deformation and lysis, %	Efficiency, %	Unsporulated, subjected to deformation and lysis, %	Efficiency, %
12	3	3	50	50	77	77	80	80
24	10	10	66	66	82	82	83	83
36	15	15	71	71	90	90	92	92
48	27	27	80	80	92	92	95	95
60	40	40	80.2	80.2	93.7	93.7	96	96
72	51	51	82	82	94	94	96	96

Table 2: The effect of solutions of different concentrations on sporulated oocysts of *Eimeria tenella in vitro*.

Discussion

Currently, for treatment of coccidiosis of chickens are used herbal remedies. Currently, the proposed use of plants with anticoccidiostatic activity as alternatives in the fight against coccidiosis [3-5,8,9,12,14,15,17,20,22]. As is known from the literature, the components included in plants have antioxidant, antiviral, antitumor, antibacterial, antiparasitic, etc. properties [6,16].

Molan and Tomas, who studied the effect of 10 and 25% green tea extracts on the sporulation of the oocysts *E. tenella, E. acervulina* and *E. maxima*, revealed that tea extract delays the sporulation by 30% [18].

Study of effect of plant complex on *E. tenella* oocysts on the example of chickens revealed that the complex consisting from mixture leaves of Azadirachta indica and of Nicotiana tabacum, of *Calotropis procera* flowers and of *Trachyspermum ammi* seeds has anticoccidial property [24]. Identification and use of drugs that prevent sporulation of oocysts is one of the most important and promising areas of in the fight against coccidiosis [5,9,12,18,20].

Coccidian oocysts re resistant to physical and chemical effects due to the presence of a two-layer protein wall [10]. Oocysts of the genus *Eimeria* are mainly composed of lipids, tyrosine- rich proteins and carbohydrates. It was

revealed wormwood destroys oocysts by inhibiting sporulation of coccidian oocysts by lysis of oocyst membranes. This proves the anticoccidial property of plant extracts.

Overall these findings about destruction and lysis of the protein wall of sporulated oocysts in Baycox solution (1ml/l) are in accordance with findings reported by Jatau., *et al* [13].

The results show that some chemical agents do not have the property of lysis of sporulated oocyst wall. The results of our research show that dose 1ml/l Baycox lead to lyses *E. tenella* oocysts wall and to the death of oocysts. Also, *A. absinthium* extract inhibits sporulation and caused lyses *E. tenella* oocysts. The research results revealed that *A. absinthium* extract can be used as a disinfectant for the prevention of eimeriosis.

Conclusion

1. It was revealed that methanol extract of wormwood a concentration of 25 mg/ml, 50 mg/ml, 100 mg/ml *in vivo* have an anticoccidial effect.
2. Revealed that a concentration 100 mg/ml led to the strongest anticoccidial effect.

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