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Influence of the efficacy of impact of extract *artemisia absinthium* on oocysts of *eimeria zuernii* in vitro

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Abstract

The paper is devoted to research of the influence of the effectiveness of impact of extract *Artemisia absinthium* on oocysts of *Eimeria zuernii* in vitro.

The results of the study revealed that *A. absinthium* extract has a coccidiostatic effect on sporulated and unsporulated *E. zuernii* oocysts, regardless of dose. *A. absinthium* extract at a dose of 100 mg / ml inhibit the vital functions of 95% of non-sporulated oocysts and 94% of sporulated of oocysts. A similar situation was observed during the incubation of oocysts at a temperature of 27 °C for 72 hours at a dose of 1 ml / l Baycox. Baycox at this dose inhibit sporulation of 97% of oocysts, and causes deformation and lysis in 96% sporulated oocysts.

It was revealed that methanol extract of wormwood (*A. absinthium*) has been an *in vitro* antieimeric effect at doses of 25 mg / ml, 50 mg / ml and 100 mg / ml. It was found that *A. absinthium* extract at a dose of 100 mg / ml to have the highest effect against sporulation oocysts of *Eimeria zuernii*.

Keywords: *Eimeria zuernii*, oocysts, *Artemisia absinthium*, extract

Introduction

Eimeriosis (coccidiosis) a disease of the digestive system caused by single-celled protozoan parasites of the genus *Eimeria*, which causes diarrhea with mass destruction of intestinal epithelial cells (Matos *et al.*, 2018; Madlala *et al.*, 2021) ^[1, 2]. The disease affects the development of animals and the loss of their weight leads to the mass, thereby leading to significant economic losses (Sarfaraz *et al.*, 2025) ^[3].

Coccidiosis is a serious threat to poultry and livestock industry. An infected animal excretes thousands of microscopic coccidian oocysts in its feces daily, leading to contamination of feed, water, and soil. The life cycle is characterized by an extended infection period and typically lasts approximately 3 to 10 days, depending on the coccidian species, host species, infectious dose of oocysts, the host's condition and age, and other factors (Gardiner *et al.*, 1998) ^[4].

Virtually all vertebrate animals serve as hosts to one or more species of protozoan parasites that cause an infection known as coccidiosis. Thousands of species of coccidia have been described in herbivorous, omnivorous, and carnivorous animals, while many others remain unnamed. Some of these species cause serious disease, whereas others have little or no clinical significance. Only a few coccidian species parasitizing ruminants are associated with severe disease. For instance, among the 13 or more species infecting cattle and bison, only *Eimeria bovis* and *Eimeria zuernii* are the primary agents of life-threatening coccidiosis, whereas some other species exhibit only moderate pathogenicity (Cicek *et al.*, 2007, Rehman *et al.*, 2011, Mundt *et al.*, 2005) ^[5-7]. *Eimeria bovis* and *Eimeria zuernii* are pathogenic species of the genus *Eimeria* that cause coccidiosis in cattle. Both species are agents of diarrheal disease, primarily affecting young animals, and are transmitted via the fecal-oral route through contaminated oocysts. Despite this, animals are often affected by simultaneous infections with multiple *Eimeria* species, both pathogenic and non-pathogenic, which significantly complicates diagnosis. The most susceptible animals are calves aged 6 to 12 months, as well as lambs and kids aged 1 to 6 months, although most clinical signs typically appear between 4 and 8 weeks of age (Keeton *et al.*, 2018) ^[8].

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Failure to comply with veterinary, sanitary-hygienic rules in livestock farms leads to the development of pathogens of diseases. Oocysts excreted from bird feces are non-sporulated and become infectious only after sporulation. Temperature, humidity, and oxygen are essential for oocysts to sporulate (Edgar, 1955) ^[9]. Ideally, oocysts sporulate for 28 to 48 hours. As a result of sporulation, 4 sporocysts are formed, each with two sporozoites. Species belonging to the genus *Eimeria* not only possess a high reproductive potential but also exhibit remarkable resistance of their sporulated oocysts to environmental factors (Kheisin, 1967) ^[10]. Non-sporulated oocysts are sensitive to environmental factors, so the absence of any of the above factors leads to the destruction of the parasite by preventing their sporulation.

Kheisin, notes that as a result of infection with *E.tenella* oocysts, 400,000 oocysts are excreted from the host (Kheisin, 1967) ^[10]. Invasion begins when oocysts secreted by infected birds enter the organism orally after sporulation, where they complete the endogenous developmental stage, and ends when the parasite leaves the host to enter the exogenous developmental stage. One of the measures to combat the disease, and almost the most important, is to prevent the exogenous development of oocysts. Different means and different chemicals are used for this purpose. Chemicals used to prevent oocysts from sporulation in the environment cause environmental problems by polluting the environment. At present, drug prevention from sporulation of oocyst is one of the most successful preventive measures (Abbas *et al.*, 2011a) ^[11]. In such cases, there is a need to develop new drugs to effectively combat resistant strains. Due to the fact that the development of new chemicals (Molan *et al.*, 2007, Molan *et al.*, 2009, Gadelhaq *et al.*, 2018) ^[12-14] and vaccines requires a lot of money, and the long-term use of newly developed drugs leads to the development of parasitic tolerance and environmental problems, increased attention is paid to the use of environmentally friendly, natural resources against coccidiosis (Noaman, 2011, Ola-Fadunsin *et al.*, 2013, Thangarasu *et al.*, 2016) ^[15-17].

The European Union's ban on the use of ionophore coccidiostatic drugs and antibiotics used to prevent coccidiosis in poultry and livestock raises the need for new, alternative means. At present, it is proposed to identify plants with coccidiostatic effect (Asghar *et al.*, 2015, Fatemi *et al.*, 2015) ^[18, 19] and use them as an alternative tool to combat coccidia (Seung *et al.*, 2007, Kostadinovic *et al.*, 2012, Emanfo *et al.*, 2017) ^[20-22]. The use of plants as an alternative to coccidiosis drugs opens up new perspectives. As known plants contain several groups of active substances, alkaloids, glycosides, tannins, saponins, flavonoids, vitamins, fatty and essential acids, various organic acids, trace elements, etc. In addition to the above, medicinal plants may contain such active components that their composition and mechanism of action have not yet been studied. Essential oils have an inhibitory effect on the activity of pathogenic microorganisms. The literature reports on the antioxidant (Ljiljana *et al.*, 2016) ^[23] anthelmintic and other properties of various plants containing essential oils (Adnane *et al.*, 2013, Mohammed *et al.*, 2021) ^[24, 25]. Medicinal plants are considered harmless in folk medicine. Indeed, some plants do not have such harmful effects even in high doses. However, plants used in the treatment of various diseases are not always harmless. There are some herbs that, if not used properly, can cause serious complications in the organism.

In this regard, the aim of the study was to estimate the effect of different doses of methanol extract of *Artemisia absinthium*

on *Eimeria zuernii* oocysts *in vitro*.

Materials and Methods

The research was conducted in the laboratory of biochemistry of parasite-host relations of the Institute of Zoology of the Ministry of Science and Education of the Republic of Azerbaijan. The fresh leaves of *A. absinthium* used in the experiments were selected from other morphological parts of the plant, washed with water and dried in the shade and outdoors for two weeks. The dried leaves of the plant are mechanically crushed into small pieces in a mortar. Soxhlet extractor was used to prepare the extract from dried leaves. For this purpose, 20 g of plant samples were taken and extracted 3 times in 500 ml of 100% methanol in 72 hours. The extract in methanol was dried in an evaporator at 40°C and diluted with TWEEN 80 (sorbitan monooleate) in a ratio of 1: 4 (extract: tween 80). The extract of the plant in methanol was filtered in a vacuum, dried and stored in a refrigerator at a temperature of 4°C. To prepare a solution of *A. absinthium* extract prepared in doses of 25, 50 and 100 mg / ml, 2.5, 5 and 10 ml of extracts were taken, respectively, and dissolved in 100 ml of water. These solutions have been used in experiments. Anticoxidiotic effect of solutions of extract *A. absinthium* of different concentrations, was evaluated by comparing the results obtained with the use of Baycox, which has a high anticoxidiotic effect. For this purpose, 1 ml of Baycox was dissolved in 1 liter of water and used in experiments. To study the effect of wormwood extracts on oocysts *in vitro*, oocysts isolated and purified from animals faeces were divided into two parts. In order to sporulate the oocysts the first solution of 2.5% potassium dichromate with oocysts in a Petri dish was placed in a thermostat at a temperature of 27-29 °C.

Temperature, oxygen, and humidity, which are important for sporulation oocyst in the thermostat, were optimal. For this purpose, the influence of wormwood extract in doses 25, 50 and 100 mg/ml and Baycox in a dose 1ml / l was revealed. Since a 2.5% solution of potassium dichromate was considered a favorable environment for the sporulation of oocysts *Eimeria*, and Baycox had a high coccidiostatic effect, both solutions were taken as a control.

Eimeria zuernii oocysts used in the studies were separated by centrifugation from fecal samples. Each 1 ml of *Eimeria zuernii* oocyst suspension contained 30,000 oocysts.

The experiments were carried out according to the following scheme. The solutions of wormwood of various concentrations (25, 50, 100 mg/ml) and Baycox solution (1 ml / l) were poured into Petri dishes in a volume of 5 ml. and then oocysts were added there. The experiments were performed at room temperature (27-29 °C) for 72 hours. Depending on the course of the experiment, oocysts were counted under a microscope x 40 lens by taking 0.001 ml of suspension oocysts every 12, 24, 36, 48, 60 and 72 hours and placing it on a glass slide, and morphological changes occurring in oocysts were studied. To assess the coccidiostatic activity of solutions, the number of sporulated, non-sporulated, deformed and lysed oocysts was determined and compared with the control group, expressed as a percentage of the total number of oocysts. The obtained results were statistically analyzed and the average value of 5 experiments was calculate.

Results

The processes of sporulation and the formation of invasive oocysts of *Eimeria* occur at different times in different regions. Variations are observed even among various

ecosystems within the same climatic zone. In fecal samples of cattle (*Bos taurus*) collected in the lowland zone, six species of the genus *Eimeria* were identified: *Eimeria auburmensis*, *E. zuernii*, *E. ellipsoidalis*, *E. zurnabadensis*, *E. subspherica*, *E. cylindrica*, and *E. bovis*.

In particular, *E. bovis* and *E. zuernii* are highly pathogenic and can cause mortality and morbidity by disturbing absorption mechanisms in calves and young animals (Cicek *et al.*, 2007, Rehman *et al.*, 2011, Mundt *et al.*, 2005) [5-7].

Sporulation of *Eimeria* oocysts was recorded in different ecosystems of the lowland zone. Experimental results demonstrated that in spring, under lowland moist pastures beneath the substrate, sporulation of *E. zuernii* occurred within three days at a temperature of 28 °C, with up to 78% of oocysts becoming invasive. Oocysts exposed in feces to direct sunlight did not undergo sporulation. In summer, at temperatures ranging from 27-38 °C, sporulation under the substrate of lowland moist pastures was completed within one day, with 82% of oocysts becoming invasive. In autumn, at temperatures of 15-21 °C, sporulation under the substrate of lowland moist pastures occurred within six days, with 60% of oocysts becoming invasive. On pastures, sporulation occurred on the sixth and seventh days, with 59% and 50% of oocysts becoming invasive, respectively.

Analysis of environmental contamination with *Eimeria* oocysts demonstrated that the sources of infection for cattle may include water, pastures, and feed contaminated with *Eimeria* oocysts.

It is known that the effect of disinfectants on the sporulation of oocysts depends on the strain of the parasite, the duration of contact of disinfectants with oocysts. Substances used against oocysts are considered effective when they penetrate the oocysts, and in that short period of time, being in contact with them, destroy them, preventing sporulation. The substances we used in these studies were in contact with *Eimeria zuernii* oocysts for 72 hours from the beginning to the end of the experiment. The study of different concentrations of the extract of *A. absinthium* and the *in vitro* effect of Baycox on oocysts shows that sporulation occurs in all concentrated solutions of the extract. If wormwood extract at a dose of 25 mg / ml prevents the sporulation of 89% of oocysts during a 12-hour incubation period, the efficiency of the drug at a dose of 50 mg / ml is 99.8%, and at a dose of 100 mg/ml-100%. During this incubation period, the efficiency of Baycox was 100% (Table 1).

As the duration of contact of the oocysts with the extract

increases, the number of sporulated oocysts also changes. The number of lysed or deformed oocysts increases depending on the duration of the extract's effect on the sporulated oocysts. In a solution of wormwood extract (at a dose of 25 mg / ml), parasite oocysts sporulate within 24 hours and the percentage of sporulated oocysts is 25%, then after 72 hours this figure rises to 40%. At a dose of 50 mg/ml of the extract, 17% of oocysts are sporulated during 72 hours of incubation, and at a dose of 100 mg / ml of the extract, 5%. During a 72-hour incubation period, 3% of oocysts were found to sporulate in 1 ml/l Baycox solution.

At a temperature of 27 °C in a 2.5% K₂Cr₂O₇ solution, which is considered the optimal medium for sporulation oocysts, 97% of oocysts sporulated after 72 hours of incubation, and only 3% of oocysts in solution were not sporulated and deformed (Table 1). Thus, it was found with an increase in the concentration of solutions prepared from wormwood extract and with an increase in the time of contact with oocysts, the number of unsporulated oocysts increases. More precisely, with an increase in the concentration of the solution, the intensity of oocyst sporulation decreases. When studying the effect of 1 ml/l solution of Baycox on oocysts, it was found that their maximum sporulation was recorded during the incubation period of 72 hours, during which sporulated oocysts accounted for 3% of total oocysts.

The results of the study showed that although *A. absinthium* extract has a coccidiostatic effect on unsporulated oocysts *in vitro*, regardless of the dose, the extract has a higher coccidiostatic effect at a dose of 100 mg / ml. At 27 °C, it showed high efficacy (95%) during the 72-hour incubation period at Baycox (97%), which was used for comparison to assess the efficacy of extract *A. absinthium*. *A. absinthium* at a dose of 25 mg/ml prevents the sporulation of 60% of oocysts, and at a dose of 50 mg/ml-83% (Table 1).

The data obtained from the study of the effect of the extract *A. absinthium* and Baycox on sporulated *Eimeria zuernii* oocysts are given in Table 2. Plant extracts have been shown to affect sporulated oocysts as well as unsporulated oocysts.

The results of the study showed that the extract of wormwood (*A. absinthium*) affects the sporulated and non-sporulated oocysts of *Eimeria zuernii*, regardless of the dose used. The extract at a dose of 25 mg/ml causes deformation or lysis of 51% of sporulated oocysts within 72 hours, at a dose of 50 mg/ml-82%, and at a dose of 100 mg/ml-94%. Baycox efficiency was found to be 96% at this time (Table 2).

Table 1: *In vitro* effects of solutions with different concentrations on unsporulated *Eimeria zuernii* oocysts

Incubation period, hours	Solutions of different concentrations in the extract of the <i>A. Absinthium</i>									K ₂ Cr ₂ O ₇ solution	Baycox, 1 ml / l					
	25 mg / ml			50 mg / ml			100 mg / ml									
	Sporulated, %	unsporulated, deformed, lysed%	Efficiency, %	Sporulated, %	unsporulated, deformed, lysed%	Efficiency, %	Sporulated, %	unsporulated, deformed, lysed%	Efficiency, %		Sporulated, %	unsporulated, %	Efficiency, %	Sporulated, %	unsporulated, deformed, lysed%	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 2: *In vitro* effects of solutions with different concentrations on sporulated *Eimeria zuernii* oocysts

Incubation period, hours	Solutions of different concentrations in the extract of the <i>A. absinthium</i>						Baykoks, 1 ml / l	
	25 mg / ml		50 mg / ml		100 mg / ml		unsporulated, deformed, lysed%	Efficiency, %
	unsporulated, deformed, lysed%	Efficiency, %	unsporulated, deformed, lysed%	Efficiency, %	unsporulated, deformed, lysed%	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

Discussion

Recently, the use of plants in the treatment of coccidiosis is proposed. Extensive research is being conducted around the world to reveal the antioxidant properties of plants (Kostadinovic *et al.*, 2012, Ljiljana *et al.*, 2016, Emanf *et al.*, 2017, Gadelhaq *et al.*, 2018, Qabaha *et al.*, 2021, Tchodo *et al.*, 2024, Maodaa *et al.*, 2024) [14, 20-23, 26-28].

It is known from the literature that plant components have antioxidant, antiviral, antitumor, antiparasitic, antibacterial, and other effects. Molan and Thomas studied the effect of 10 and 25% green tea extract on the sporulation of *E.tenella*, *E.acervulina* and *E.maxima* oocysts and showed that tea extract slows down the process of sporulation of unsporulated oocysts by 30% (Molan *et al.*, 2007) [12]. The effect of plant complexes on *E.tenella* oocysts on chickens was observed and it was determined that complex mixtures made from leaves of *Azadirachta indica* and *Nicotiana tabacum*, flowers of *Calotropis procera*, seeds of *Trachyspermum ammi* plant, hot pepper extract have anticoccidial effect (Zaman *et al.*, 2012) [29].

Detection of drugs that prevent the process of sporulation and their application is one of the most promising tools in the implementation of preventive measures against coccidiosis (Guimaraes *et al.*, 2007, Molan *et al.*, 2007, Noaman, 2011, Asghar *et al.*, 2005, Fatemi *et al.*, 2015, Gadelhaq *et al.*, 2018) [12, 14, 15, 18, 19, 30].

Boyko and *et al.* researched the influence of essential oils from 14 species of plants (*Piper cubeba*, *Cananga odorata*, *Pelargonium graveolens*, *Citrus sinensis*, *Eucalyptus globulus*, *Lavandula angustifolia*, *Picea abies*, *Citrus paradisi*, *Pterocarpus santalinus*, *Abies sibirica*, *Juniperus communis*, *Melaleuca alternifolia*, *Syzygium aromaticum* and *Cinnamomum verum*) on the process of sporulation of *Eimeria magna*. In the experiment we used 0.5% emulsion of oil during 72 hours, oocysts of *E.magna*. Essential oil from *C. verum* exerted the strongest influence on oocysts of *E.magna*, causing death to 100% of oocysts during 72 hours exposure. About 54% mortality of partially sporulated oocysts was observed during the influence of emulsion of essential oil of *S.aromaticum*, while no sporulated oocysts were recorded. Many of the tested essential oils can delay the sporulation of *E. magna* oocysts (*P. abies*, *C. paradisi*, *P. santalinus*, *A. sibirica*, *J. communis*, *M. alternifolia*). Oils from *P. cubeba*, *C. odorata*, *P. graveolens*, *C. sinensis*, *E. globulus*, *L. angustifolia* had no effect on sporulation of *E. magna* (Boyko *et al.*, 2021) [31].

Coccidial oocysts are highly resistant to physical and chemical effects because they have a double-layered protein layer. *Eimeria* oocysts are mainly composed of lipids, tyrosine-rich proteins and carbohydrates (Ferguson *et al.*, 2003) [32]. It has been found that wormwood extract destroys the walls of oocysts by lysing them, preventing them from sporulation, and even some oocysts cannot be recorded under a microscope. This proves that *A.absinthium* extracts have an anticoccidial effect. The extract affects the vital activity of oocysts, weakening their sporulation process. Data on the destruction and lysis of the protein layer of sporulated oocysts by 1 ml/ l solution of Baycox were obtained from Jatau *et al.* (Jatau *et al.*, 2013) [33] differs from our data. They show that some chemicals do not have the ability to lyse the walls of sporulated oocysts. The results of our study show that a solution of Baycox in a dose of 1 ml/l lyses the walls of *Eimeria zuernii* oocysts and promotes their destruction. *A.absinthium* extract, like Baycox, prevents sporulation of

Eimeria zuernii oocysts and causes their wall to lysis. The results of our study show that *A. absinthium* extract can be used as a disinfectant for the prevention of eimeriosis.

Conclusion

Methanol extract of wormwood (*A.absinthium*) at doses of 25 mg/ml, 50 mg/ml and 100 mg/ml has been shown to have an *in vitro* anticoccidial effect.

The extract of *A. absinthium* at a dose of 100 mg/ml was found to have the highest effect against sporulation of *Eimeria zuernii* oocysts.

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Conflict of interest

The author has no conflict of interests to declare regarding the publication of this paper.

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