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In vitro Anticoccidial Activity of *Artemisia absinthium* on *Eimeria* spp Oocysts of Broiler Chickens

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Abstract

COCCIDIOSIS presents significant challenges for the poultry industry, resulting in substantial economic losses. The objective of this study was to assess the in vitro efficacy of the aqueous extract of *Artemisia absinthium* on *Emeria spp*. oocysts isolated from broiler chicken feces. Oocysts (mean initial number per well: 631 ± 26) were distributed into multiwell plates and treated with increasing concentrations of *Artemisia absinthium* infusion (10%, 5%, 2.5%, 1.25%, 0.62%, 0.31%, 0.15%, 0.07%), then incubated at 28°C for 24, 48, 72, and 96 hours. Potassium dichromate was used as the positive control. Oocyst viability and sporulation were monitored at 24-hour intervals using established counting methods. After 96 hours, the 10% *Artemisia absinthium* treatment achieved an $87.9 \pm 06.5\%$ reduction in oocyst count ($p < 0.001$). The reduction in the number of oocysts at 5%, 2.5% and 1.25% was also significantly greater than at lower concentrations ($p < 0.001$). Sporulation was markedly inhibited, ranging from 91.7% to 100% depending on concentration ($p < 0.001$), whereas the sporulation rate for the positive control (potassium dichromate) was $78.4 \pm 1.8\%$ versus 0–6.5% in treated groups ($p < 0.001$). Statistical modeling using a generalized linear and mixed model confirmed that the efficacy of *Artemisia absinthium* increased both with extract concentration and with incubation time ($p < 0.001$). The results showed a clear and consistent trend in both oocyst reduction and inhibition of sporulation as exposure parameters increased. Taken together, these results indicate that aqueous *Artemisia absinthium* extract displays a strong in vitro anticoccidial effect, demonstrating significant reduction in oocyst viability and preventing sporulation. *Artemisia absinthium* thus represents a promising natural antiparasitic candidate for sustainable poultry coccidiosis management. However, further in vivo investigations are necessary to confirm its effectiveness and safety under field conditions.

Keywords: *Artemisia absinthium*; oocysts; *Emeria spp*; broiler chicken; *In vitro*.

Introduction

Coccidiosis is a major parasitic disease impacting global poultry production due to the economic losses caused by morbidity, reduced growth performance, and high mortality, especially in broiler chickens [1,2]. Infection with several species of the genus Eimeria results in intestinal lesions, growth retardation, and decreased feed conversion efficiency [3,4].

In Algeria, coccidiosis remains a persistent problem in all poultry farms, with a high prevalence observed notably in the Blida region [5]. In the context of poultry farming, the use of medicines plays a pivotal role in health and welfare. Farmers employ a range of drugs to prevent and treat disease, control parasites and promote animal growth [6].

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Nevertheless, the misuse of pharmaceuticals in industrial poultry farming can lead to many issues, including the development of drug resistance, the presence of residues in meat, eggs and processed animal products, and thus concerns regarding food safety [7]. Moreover, vaccination against coccidiosis, although effective, remains rarely implemented in intensive systems due to its prohibitive cost at the farm level [3,4].

Substantial efforts have been exerted to investigate alternative methods of treating coccidiosis, with the aim of effectively combating this disease and improving the zootechnical performance of chickens [8]. Plants contain a vast array of compounds, forming an extensive reservoir of chemical diversity with a wide range of biological activities [9]. This justifies the growing number of studies devoted to plant species that could be potential natural sources of medication [10], including the genus Artemisia. Artemisia absinthium contains bioactive molecules such as absinthin and thujone, which have demonstrated significant antiparasitic effects against a range of animal and protozoan parasites. In addition, the aqueous extracts of Artemisia absinthium show high antioxidant activity and further pharmacological actions supporting the control of coccidiosis and enhancing the health of poultry [11,12]. Accumulating evidence confirms that this plant's phytochemical diversity, particularly its polyphenols and terpenes, plays a central role in disease prevention, justifying its inclusion in contemporary poultry research programs [13].

A recent systematic review and meta-analysis conducted by our team [5] highlighted the promising potential of Artemisia absinthium for the control of coccidiosis in broiler chickens. The results obtained prompted the present study, which aims to evaluate in vitro the effect of the aqueous extract of Algerian *Artemisia absinthium* on the number and sporulation of Eimeria *spp* oocysts isolated from broiler chickens

Material and Methods

Preparation of the inoculum

Eimeria spp. oocysts were obtained from broiler droppings collected from a variety of farms. The oocysts were purified by flotation in a saturated salt suspension. Subsequently, they were enumerated on a MacMaster cell, washed with distilled water and centrifuged at 4600 rpm for five minutes. The pellets were then mixed with 2.5% potassium dichromate (K_2Cr_2O7, Sigma-Aldrich, Saint-Louis, MO, USA) and stored at 4°C.

Preparation of the aqueous extract and dilutions

The leaves and stems were pulverised. 1000 mL of boiling water were added to 100 g of powder. The preparation was then allowed to infuse for a period of three hours, after which it was filtered. The solution was concentrated to 10% and stored at 4°C as a "stock solution" for later use. The following dilutions were prepared: 5%, 2.5%, 1.25%, 0.62%, 0.31%, 0.15%, 0.07%.

Experimental design

A cohort of normal (intact), washed, and quantified oocysts (mean initial number per cohort = 631 ± 26) was retrieved from refrigeration and allocated into distinct wells of a Costar® cell culture plate. Each well was supplemented with 1 mL of Artemisia absinthium infusion across a concentration gradient (10%, 5%, 2.5%, 1.25%, 0.62%, 0.31%, 0.15%, and 0.07%) and subsequently incubated at 28 °C for intervals of 24, 48, 72, and 96 hours. To ensure statistical robustness and limit variability between groups, the experimental design incorporated three replicates per treatment at each incubation time point. Corresponding positive controls using potassium bichromate were prepared under identical conditions. This protocol conforms to methodologies recommended by Habibi et al. [14], who advocate for the inclusion of at least 3–5 replicates per treatment group to facilitate reliable quantitative comparison in oocyst inhibition assays.

Oocysts exhibiting damage or deformation were considered lost. The reduction rate in the number of oocysts (96 hours post-incubation) was determined using the following formula for the mean number of oocysts for each treatment [15]:

$$Oocyst\ reduction\ (\%) = \frac{Mean\ oocyst\ number\ in\ control\ group - Mean\ oocyst\ number\ in\ treatment\ group}{Mean\ oocyst\ number\ in\ control\ group} \times 100$$

Oocysts were considered sporulated in the presence of sporocysts, regardless of their shape, size, or number of sporocysts. Sporulated and non-sporulated oocysts number were counted (96 hours post incubation), and sporulation and relative sporulation inhibition rates were calculated using the equations suggested previously [16,17],

$$Sporulation\ Rate\ (\%) = \frac{sporulated\ oocysts}{total\ number\ of\ oocysts} \times 100$$

$$Relative\ Inhibition\ (\%) = 100 - 100 \times \frac{exposed\ groups}{negative\ control}$$

The oocysts treated with the different concentrations were washed and reconstituted in potassium dichromate and incubated at 28°C for a further 96 h to check for sporulation.

Data analysis

All statistical analyses were conducted using R software (version 4.2.2, Development Core Team, R Foundation for Statistical Computing, 2022). The values were considered statistically significant when the p-value was ≤ 0.05. The mean and standard deviation were calculated for the number of treated,

sporulated, non-sporulated, and degenerated oocysts at 0 h, 24 h, 48 h, 72 h, and 96 h. The overall impact of the treatments was quantified using a one-way analysis of variance (ANOVA) followed by Tukey's test. A generalized linear and mixed model (GLM or GLMM) with a negative binomial link was employed, utilizing the glmer function of the lme4 package. A Pearson chi-square statistic was employed to ascertain the absence of overdispersion in the models. A generalized linear mixed model (GLMM) with a negative binomial distribution was employed to investigate the influence of time interval periods on the number of oocysts. This model integrated time interval as a fixed effect and individuals as a random effect. Additionally, the potential effects of time intervals and *Artemisia absinthium* concentration on oocyst numbers were examined. The focus was on the interaction between "time intervals" and "*Artemisia absinthium* concentrations" as potential predictors (fixed effect). A GLM with a negative binomial link was conducted to (1) ascertain whether the product concentration influenced the number of oocysts (with the product concentration serving as the explanatory variable and the number of oocysts as the outcome variable) and (2) investigate the effect of the treatment concentration of *Artemisia absinthium* on the proportion of sporulation. The Wilcoxon rank sum test was performed to assess the impact of the two treatments, potassium dichromate and *Artemisia absinthium*, on the proportion of oocysts that underwent sporulation at 96 hours.

Ethical Statement

All the animal studies were conducted with the utmost regard for animal welfare, and all animal rights issues were appropriately observed. No animal suffered during the course of the work. All the experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA. 14).

Results

Evaluating the effect of Artemisia absinthium infusion on the morphology of Eimeria spp Oocysts

The results of microscopic observation before treatment and in the positive control (potassium dichromate) demonstrated the presence of structurally intact oocysts in the non-sporulating and sporulating *Eimeria spp*. (Fig. 1).

Following treatment with *Artemisia absinthium* infusion at concentrations of 10%, 5% and 2.5%, microscopic observation revealed the destruction of the oocysts, resulting in a reduction in the number or deformation of the external or internal structures of the oocysts, which were also considered lost (Fig. 2).

Evaluation of the in vitro effect of different concentrations of Artemisia Absinthium on oocysts reduction and sporulation inhibition

The data presented in Table 1 demonstrate the impact of different concentrations of *Artemisia absinthium* (10%, 5%, 2.5%, 1.25%, 0.62%, 0.31%, 0.15%, 0.07%) on oocysts of *Eimeria spp.*

The count data, characterized by overdispersion and correlated observations, were analyzed using a generalized linear mixed model (GLMM) with a negative binomial distribution. This model is appropriate for managing variance larger than the mean and repeated measures inherent in parasitological data. The analysis revealed a significant effect of Artemisia absinthium infusion treatment on reducing oocyst counts ($p < 0.001$) and marked inhibition of sporulation. The effect was dose-dependent, with a progressive decrease in oocyst numbers corresponding to increasing extract concentrations. In comparison to the control (potassium dichromate), the reduction in the number of oocysts was found to be significantly higher following treatment with *Artemisia absinthium*. The reduction in the number of oocysts at a concentration of 10% was found to be significantly higher than at 5% ($p < 0.001$), 2.5% ($p=0.002$), 1.25% ($p < 0.001$), 0.62% ($p < 0.001$), 0.31% ($p < 0.001$), 0.15% ($p < 0.001$), and 0.07% ($p < 0.001$), respectively. In a similar manner, the reduction in the number of oocysts at a concentration of 5%, 2.5% and 1.25% was significantly higher than at 0.62%, 0.31%, 0.15% and 0.07%.

The percentage of sporulation observed in wells treated with potassium dichromate ($78.4 \pm 1.8\%$) was significantly higher than those treated with *Artemisia absinthium* ($p < 0.001$). The absence of sporulation was observed in wells treated with a concentration of 10%, 5%, 2.5% and 1.25%, while the lowest sporulation percentages were recorded with *an Artemisia absinthium* concentration of 0.62% (3.28%), 0.31% (4.06%), 0.07% (6.46%) and 0.15% (6.25%), resulting in the highest relative sporulation inhibition (RI) percentages (from 91.73% to 100%).

Oocysts that had been treated with *Artemisia absinthium,* washed and then reinfused in potassium dichromate showed no sporulation after a further 96 hours of incubation. This demonstrates the definitive effect of the infusion treatment on sporulation.

Effect of time interval periods on the number of oocysts

Our results indicated that as time progressed, the number of oocysts tended to decrease significantly across the different time intervals (Fig. 3). Data of estimating the proportional change in the number of oocysts as a function of time intervals revealed that at:

-24h, proportional change = exp (-0.3797) - 1 ≈ -0.3106, meaning that there was an expected significant decrease (p = 0.035) of around 31.06% in the number of oocysts compared with the initial number.

-48h, proportional Change = exp (-0.5511) - 1 ≈ -0.4174, meaning that there was an expected highly significant decrease (p =0.002) of around 41.74% in the number of oocysts compared with the initial number

-72h, proportional Change = exp (-0.7447) - 1 ≈ -0.5198, meaning that there was an expected very highly significant decrease ($p < 0.001$) of around 51.98% in the number of oocysts compared with the initial number

-96h, proportional Change = exp (-0.7888) - 1 ≈ -0.5462, meaning that there was an expected very highly significant decrease ($p < 0.001$) of around 51.98% in the number of oocysts compared with the initial number.

Effect of concentrations of Artemisia absinthium infusion on number of oocysts

The treatment concentration had a negative effect on the number of oocysts (Fig. 4), and the estimated *Artemisia absinthium* concentration coefficient was calculated to be -0.11702 (coefficient exp (-0.11702)). This showed that a one-unit increase in the treatment concentration was associated with an expected decrease of approximately 0.8895674 in the number of oocysts.

Effects of time intervals and concentration of Artemisia absinthium infusion on the number of oocysts

Interaction effects involving time intervals and *Artemisia absinthium* infusion concentration were the most important factors influencing the decrease in oocyst numbers ($p < 0.001$). The results obtained revealed a clear trend towards a decrease in oocyst numbers as time intervals lengthened and product concentration varied (Fig. 5). The largest significant negative effect (p = 0.041) on oocyst numbers was observed at 96h.

The results of estimating the proportional change in the number of oocysts as a function of time intervals and infusion concentration showed that: proportional Change = (exp(coefficient) - 1) at 24h, 48h, 72h, 96h were: -0.1259, -0.1635, -0.2213, -0.2329, respectively. This means that when the time intervals 24h,48h,72h,96h and the product concentration increased simultaneously by one unit, a decrease of about 12.59%, 16.35%, 22.13%, and 23.29%, respectively, in the number of oocysts compared to the initial number was expected.

Effect of Artemisia absinthium concentration on proportions of sporulation

The comparison between potassium dichromate and *Artemisia absinthium* treatments revealed a highly significant difference (p = 0.0008) in terms of changes in oocyst proportions at 96 hours (Fig. 6). The proportion of sporulation in the presence of the *Artemisia absinthium* infusion treatment was found to be significantly lower (coefficient exp (-4.82276), p=0.035) than in the presence of potassium dichromate, with a value of approximately 0.0084. This indicates that sporulation was approximately 115 times lower in the *Artemisia absinthium* infusion treatment than in the potassium dichromate.

Discussion

Our results demonstrated potent in vitro anticoccidial activity of the aqueous extract of *Artemisia absinthium*, with an 87.9% reduction in Eimeria oocyst count and near complete inhibition of sporulation, confirming its strong parasiticidal potential. This is consistent with Hezil et al. [18], whose systematic review and meta-analysis established Artemisia absinthium extracts as effective in reducing parasite loads and improving broiler health outcomes. Laboratory studies have similarly demonstrated that Artemisia induces lysis of oocysts and inhibits sporulation, critical steps in the *Eimeria* life cycle [2]. Beyond poultry, Artemisia exhibits broad-spectrum antiparasitic effects, including activity against *Trichinella spiralis* and *Haemonchus contortus* [19,20], supporting its multimodal therapeutic potential.

The inhibition of sporulation represents a pivotal criterion for assessing an anticoccidial product's efficacy [17]. Our findings concord with those of Zaman et al. [21], who observed a dose-dependent inhibitory effect of aqueous-methanolic *Artemisia absinthium* extract on *Eimeria tenella* sporulation, and with Habibi et al. [14], who reported a 22.5% sporulation inhibition rate after 72 hours with six different plant extracts including *Artemisia absinthium*. Other *Artemisia* species such as A. annua also demonstrated similar inhibitory effects on Eimeria spp. sporulation [22].

The anticoccidial effects are predominantly attributed to *Artemisia*'s bioactive constituents, especially absinthin and thujone. Absinthin, a sesquiterpene lactone, disrupts enzymatic pathways essential for oocyst development and sporulation, likely via mitochondrial interference and oxidative metabolism impairment in parasites [23]. Thujone, a monoterpene ketone, acts neurotoxically through modulation of GABA type A receptors, inhibiting chloride channels and causing parasite neuronal signaling disruption, oxidative stress, and oocyst inactivation [24,25]. These findings support a multifaceted mode of action targeting diverse parasite biological pathways.

Cumulative investigations by Kostadinović et al. [23] illustrate that both extracts and essential oils of

Artemisia absinthium mitigate parasitic burdens and oxidative damage in avian hosts, underscoring their promise as natural, sustainable anticoccidial agents. Microscopic examinations confirm deformation and lysis of *Eimeria* oocysts following treatment.

Notably, the efficacy of *Artemisia absinthium* extends to *in vivo* contexts, where its effects on oocyst excretion are observed when administered in various forms—essential oil, ethanolic, and methanolic extracts—through feed or drinking water [14,23-25]. Additionally, inhibitory effects on Eimeria spp. oocysts have been documented in goats and rabbits treated with *Artemisia absinthium* [26,27]. Other genus members, such as *Artemisia herba alba* and *Artemisia afra*, also demonstrate reductions in Eimeria tenella oocysts [28].

From a practical standpoint, dietary supplementation with *Artemisia absinthium* powder improves broiler performance metrics, promotes immune organ development, and enhances intestinal morphology [29]. Nonetheless, these promising in vitro and initial in vivo results require validation through rigorous, controlled studies defining dosage, safety profile, and efficacy. Potential interactions with feed additives or coccidiostats must also be elucidated to harness possible synergistic effects or avoid antagonisms.

Beyond direct parasiticidal activity, *Artemisia* extracts modulate the gut microbiome and host immune responses, increasingly recognized as central in controlling subclinical infections and maintaining gut health [13]. Phytochemicals like tannins and saponins present in *Artemisia* and related plants additionally exert antimicrobial and immunomodulatory influences, amplifying host resistance against Eimeria infections [18].

Finally, integrating *Artemisia absinthium* into integrated pest management frameworks offers a natural, holistic strategy to reduce reliance on synthetic anticoccidials, mitigate emergence of resistant *Eimeria* strains, and enhance consumer trust via lowered chemical residues [30]. Future investigations should delve further into the immunomodulatory and microbiome-related effects, alongside assessing commercial feasibility to advance sustainable, food-safe poultry production aligned with environmental stewardship.

Conclusion

The aqueous extract (infusion) of *Artemisia absinthium* exhibits significant anticoccidial potential, effectively inhibiting Eimeria oocyst sporulation, reducing oocyst counts, and damaging the parasites. This antiparasitic efficacy, well-supported by numerous in vitro and in vivo studies, highlights *Artemisia absinthium* as a promising natural alternative for coccidiosis control in poultry. The multifaceted bioactive compounds in this plant act on various stages of the parasite's life cycle, contributing to its strong parasiticidal effects. While these encouraging findings underscore its potential, further rigorous in vivo trials are essential to confirm optimal dosing, safety, and practical application in sustainable poultry production systems.

Informed Consent Statement

Not applicable

Competing Interests

The authors declare no conflict of interest related to this research.

Author Contribution Statement

All authors contributed to every stage of the study, from its design to the preparation of the manuscript.

TABLE 1. *In vitro* effects of different concentrations of *Artemisia absinthium* on *Eimeria spp* oocyst reduction and sporulation dynamics within 96 hours of incubation.

Compound	Concentration (%)	Oocysts Reduction (%)	Sporulation Dynamic	
			SP (%)	IR (%)
Control (potassium dichromate)	**2.5**	00.00 ± 0.00 [a]	78.41±01.80 [a]	00.00±0.00
Artemisia absinthium Infusion	**10.00**	87.95±06.50 [e]	00.00±0.00 [e]	100
	5.00	51.00±22.20 [b]	00.00±0.00 [e]	100
	2.50	45.70±18.60 [c]	00.00±0.00 [e]	100
	1.25	39.11±17.51 [d]	00.00±0.00 [e]	100
	0.62	11.30±04.91 [f]	03.28±0.21 [c]	95.52
	0.31	08.53±04.71 [g]	04.06±1.42 [c]	94.34
	0.15	05.9±03.40 [h]	06.25±1.15 [c]	91.23
	0.07	0**5.5**±03.52 [i]	06.46±0.80 [c]	91.73

SP (%) : sporulation rate; IR (%): Relative inhibition rate; Means followed by the same letter within a column are not significantly different ($p > 0.05$), whereas different letters indicate significant differences ($p < 0.05$).

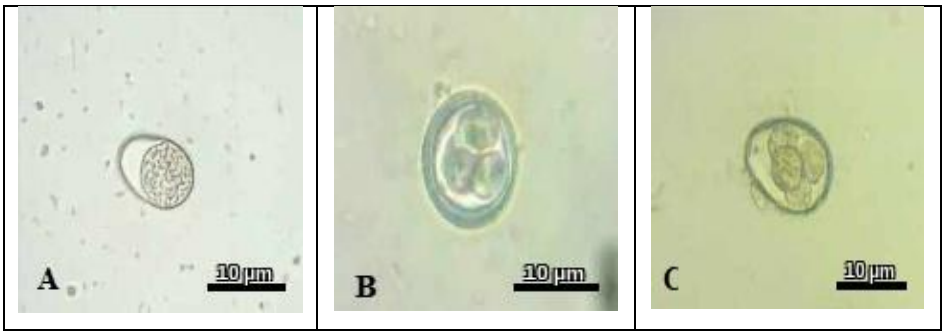

Fig. 1. Intact *Eimeria spp* oocysts, A: non-sporulated oocysts, B and C: Sporulated *Eimeria spp* oocysts.

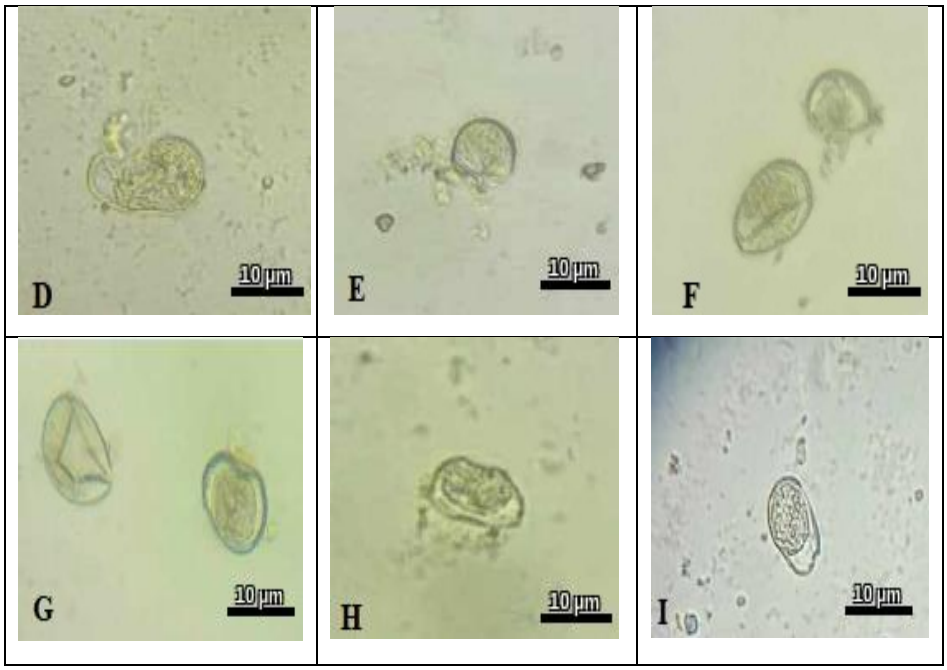

Fig. 2. *EEimeria spp* oocysts after treatment with *Artemisia absinthium* infusion.D: irregular oocyst shape, E: broken unsporulated oocyst, F: oocyst having lost its wall, G, H and I: destruction and shrinkage of some sporocysts.

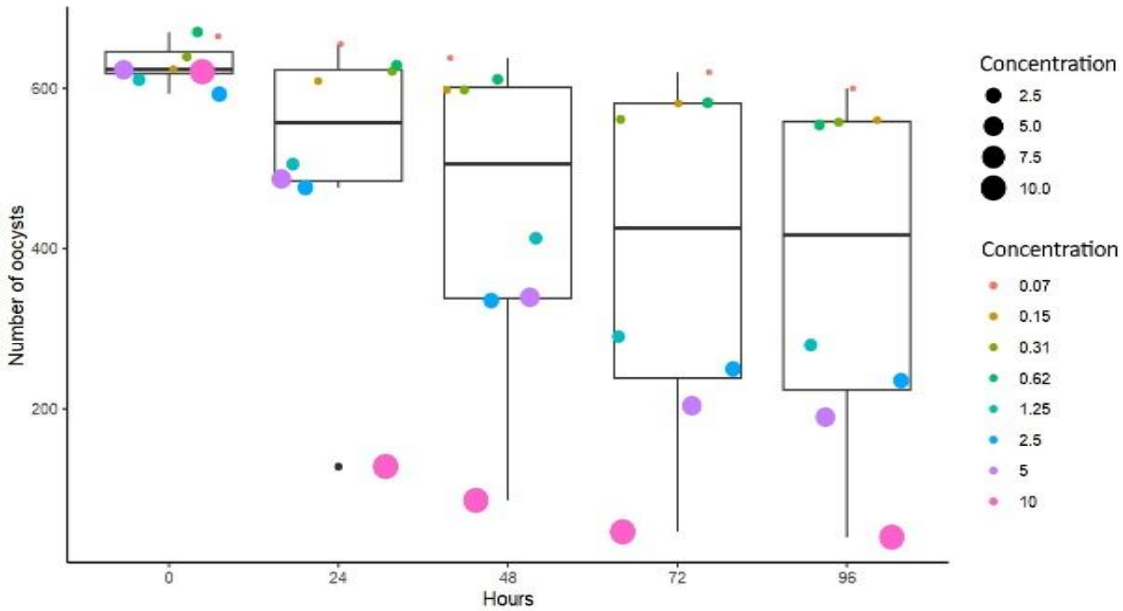

Fig. 3. Variations in oocyst reduction as a function of time and different *Artemisia absinthium* infusion concentrations

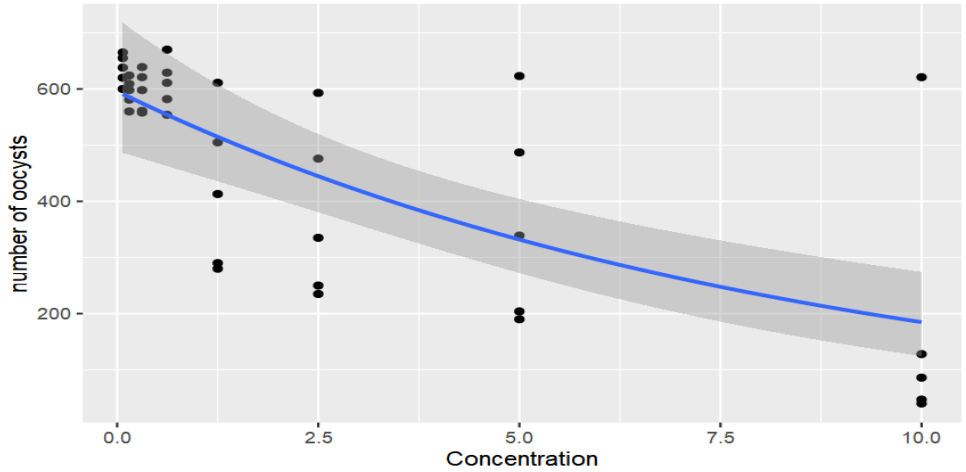

Fig. 4. Effect of concentrations of *Artemisia absinthium* infusion on oocysts number

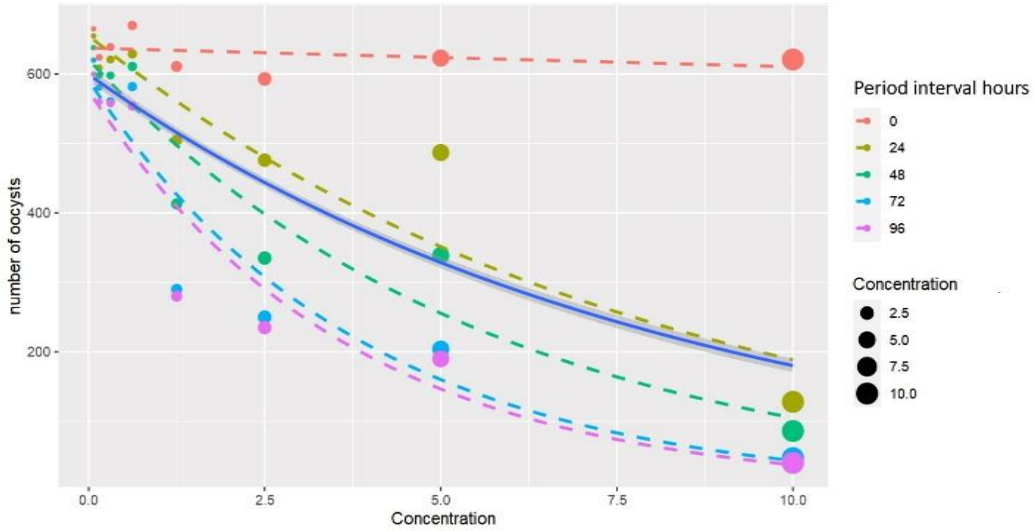

Fig. 5. Simultaneous effect of time intervals and concentration of *Artemisia absinthium* infusion on oocyst numbers

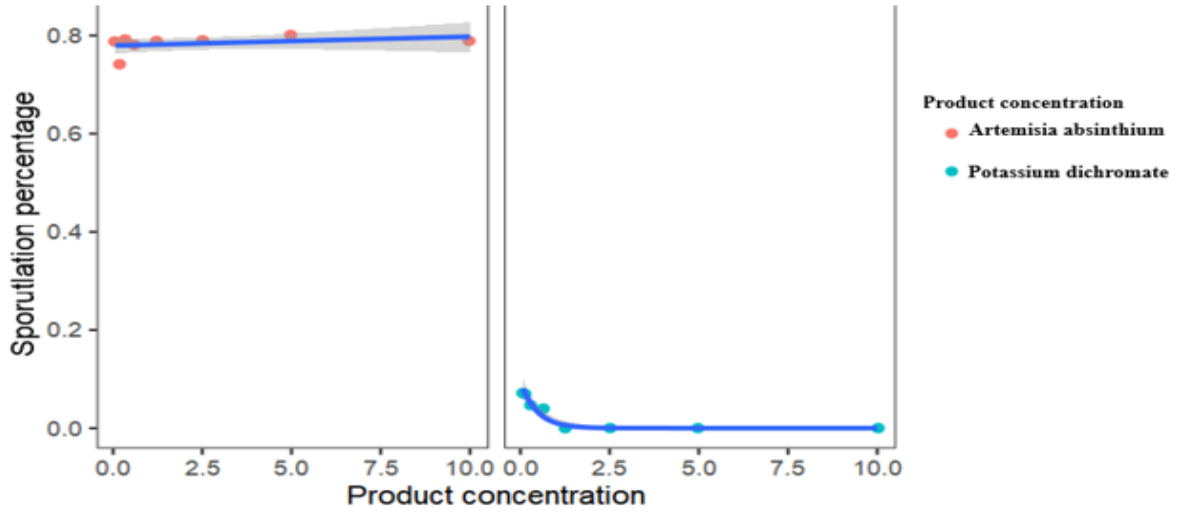

Fig. 6. Effect of *Artemisia absinthium* concentration and potassium dichromate on sporulation proportions

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النشاط المضاد للإيميريا في المختبر لنبات الشيح (Artemisia absinthium) على بويضات (Oocysts) الإيميريا Eimeria spp المعزولة من دجاج التسمين.

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الملخص:

يمثل داء الكوكسيديا (Coccidiosis) أحد أهم التحديات التي تواجه صناعة الدواجن، لما يسببه من خسائر اقتصادية معتبرة. تهدف هذه الدراسة إلى تقييم الفعالية في المختبر للمستخلص المائي لنبات الشيح (Artemisia absinthium) ضد أوسيستات (Oocysts) طفيليات الإيميريا Eimeria spp المعزولة من براز دجاج التسمين.

تم توزيع الأوسيستات (المتوسط الابتدائي لعددها في البئر الواحدة: 631 ± 26) في صفائح متعددة الآبار، ثم عُولجت بتراكيز متزايدة من مستخلص الشيح المائي (10%، 5%، 2.5%، 1.25%، 0.62%، 0.31%، 0.15%، 0.07%)، واحتضنت عند حرارة 28 درجة مئوية لمدة 24، 48، 72 و96 ساعة. استُخدم ثنائي كرومات البوتاسيوم كمجموعة ضابطة إيجابية. تمت متابعة حيوية الأوسيستات وعملية التبوغ (sporulation) على فترات زمنية قدرها 24 ساعة باستعمال طرق العد القياسية.

بعد 96 ساعة، أظهر تركيز 10% من المستخلص المائي لـ Artemisia absinthium انخفاضًا بنسبة 87.9 ± 6.5% في عدد الأوسيستات ($p < 0.001$). كما كان الانخفاض في عدد الأوسيستات عند التراكيز 5%، 2.5% و1.25% أعلى بكثير مقارنة بالتراكيز الأدنى ($p < 0.001$). لوحظ تثبيط واضح لعملية التبوغ، تَراوحَ بين 91.7% و100% حسب التركيز ($p < 0.001$)، في حين بلغت نسبة التبوغ في العينة الضابطة (ثنائي كرومات البوتاسيوم) 78.4 ± 1.8% مقابل 0–6.5% في المجموعات المعالجة ($p < 0.001$).

أكد النمذجة الإحصائية باستخدام النموذج الخطي العام المختلط أن فعالية مستخلص Artemisia absinthium ازدادت مع كل زيادة في تركيز المستخلص ومدة الاحتضان ($p < 0.001$). أظهرت النتائج اتجاهًا واضحًا ومتسقًا في خفض عدد الأوسيستات وتثبيط التبوغ مع زيادة مدة التعرض والتركيز.

تشير هذه النتائج مجتمعة إلى أن المستخلص المائي لنبات Artemisia absinthium يمتلك نشاطًا مضادًا قويًا للكوكسيديا في الوسط المخبري، إذ يُحدث انخفاضًا ملحوظًا في حيوية الأوسيستات ويمنع عملية التبوغ. وبناءً على ذلك، يمكن اعتبار مستخلص نبات الشيح Artemisia absinthium مرشحًا طبيعيًا واعدًا كعامل مضاد للطفيليات، في إطار إدارة مستدامة لمرض الكوكسيديا عند الدواجن. ومع ذلك، تبقى هناك حاجة لإجراء دراسات سريرية إضافية لتأكيد فعاليته وسلامته تحت الظروف الحقلية.

الكلمات الدالة: الشيح (Artemisia absinthium)، الأوسيستات (Oocysts)، الإيميريا Eimeria spp، دجاج التسمين، المختبر in vitro.