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# *IN-VITRO* ANTICOCCIDIAL EVALUATION OF *CITRUS SINENSIS* ESSENTIAL OIL AGAINST *EIMERIA* OOCYSTS

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## ABSTRACT

Poultry coccidiosis has been conventionally controlled by various anticoccidial chemicals. But, due to multiple issues associated with chemicals, need for safe alternates is crucial and essential oils are among the best candidates. Hence, the current *in-vitro* research was conducted to examine the anticoccidial potential of *Citrus sinensis* essential oil against mixed species of *Eimeria* oocysts. In this experiment, the anticoccidial activity was estimated by sporulation inhibition method. Eight groups were made based on different concentrations of *C. sinensis* essential oil and other control chemicals. The results revealed a dose-dependent response with maximum sporulation inhibition activity at the highest concentration of the *C. sinensis* essential oil. Thus, this essential oil is proved to have the potential for its application in control of coccidiosis.

***Keywords*:** Coccidiosis, Anticoccidials, Essential oil, *Citrus sinensis*, *In-vitro*

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## 1. INTRODUCTION

Coccidiosis is a heavily fatal disease of poultry caused by *Eimeria* species causing significant economic losses (Abbas et al. 2017a). It is a parasitic disease that infects the intestines of the birds and is marked by enteritis and bloody diarrhea (Bachaya et al. 2012). Various *Eimeria* species that infect the poultry include *Eimeria mitis*, *E. brunette*, *E. tenella*, *E. acervulina*, *E. maxima* and *E. necatrix* (Khater et al. 2020). The disease starts with the ingestion of sporulated oocysts and results in diarrhea, enteritis, emaciation, poor growth, ruffled wings, high mortality and morbidity in the flock (Abbas et al. 2020; Zhang et al. 2020). Poor management, high stocking density and wet litter act as predisposing factors to this disease (Khater et al. 2020). This infection weakens the immune system of the infected birds that leads towards the secondary infections (Ritzi et al. 2016).

There are many strategies towards the control of poultry coccidiosis. However, the use of synthetic chemicals as anticoccidial drugs in feed and water is the most practiced way of controlling the infection (Khater et al. 2020). But these anticoccidial drugs are now losing their effectiveness due to the resistance development in *Eimeria*. Moreover, due to harmful effects on public health, the use of these drugs is also being reduced (Abbas et al. 2017a). Other prominent control method being practiced is vaccination. It is an expensive method with additional risk of vaccine failure which may lead to drastic outcomes (Chapman 2014). However, there is evidence of improved response when vaccination was used in combination with botanicals (Ritzi et al. 2016). Botanicals may be dried powders, aqueous extracts, ethanolic extracts or essential oils (Murthy et al. 2012).

Essential oils are extensively known for their anticoccidial, antifungal, antibacterial, antiviral, antiparasitic and antioxidant properties (Giannenas et al. 2003; Özcan et al. 2006; Ma and Yao 2020; Lahlou et al. 2021; Yu et al. 2021). These biological properties are attributed to compounds like flavonoids, polysterols, tocopherols and polyphenols present in essential oils (Abbas et al. 2013; Jorge et al. 2016). *Citrus sinensis* essential oil also contains large amount of these bioactive compounds like tocopherols, polysterols and phenolic compounds (Jorge et al. 2016). Moreover, *C. sinensis* oil has also been proved to have antimicrobial, antifungal, insecticidal, anti-inflammatory and anti-cancer activities (Kumar et al. 2012; Murthy et al. 2012; Pendleton et al. 2012; Yang et al. 2017). But, so far, *C. sinensis* oil has not been investigated for its anticoccidial effect. Hence, this study was designed for the investigation of anticoccidial activity of *Citrus sinensis* essential oil using *in-vitro* method.

## 2. MATERIALS AND METHODS

### 2.1. Essential Oil

Essential oil of *C. sinensis* was purchased from the market of Faisalabad (Pakistan). Six (volume by volume) concentrations of the oil were made in Dimethyl sulfoxide as 10, 5, 2.5, 1.25, 0.625 and 0.31%.

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### 2.2. Collection of *Eimeria* Oocysts

The guts of the chickens that were suspected of coccidiosis were collected from the local market and farms. The guts were opened, and the contents of the guts were observed under microscope. The contents that were confirmed to have coccidial oocysts were isolated and preserved in 2.5% Potassium dichromate solution. For the isolation of the sporulated oocysts, the methodology followed was described by Ryley et al. (1976).

### 2.3. *In-vitro* Experiment

The *in-vitro* experiment involved sporulation inhibition assay for estimation of anticoccidial potential of *C. sinensis* essential oil. For this experiment, eight groups were made named as Group A to H. First six groups referred to essential oil concentrations with A denoting 10% concentration while F denoting the respective lowest 0.31% concentration. The groups G and H were the control groups having Dimethyl sulfoxide and Potassium dichromate solutions respectively. The oocysts were exposed to the respective compounds and concentrations and incubated for two days at a temperature of 25–29°C. An air pump was used to aerate the oocysts. The sporulated oocysts were rinsed twice in tap water at the end of the incubation period and then refrigerated at 4°C before being counted. The percent sporulation was estimated.

### 2.4. Statistical Analysis

The results were statistically analyzed using ANOVA and the results were considered significant at $P<0.05$.

## 3. RESULTS AND DISCUSSION

Among the various diseases of poultry, coccidiosis is a major parasitic disease having considerable economic importance (Chapman 2014). Its chemoprophylaxis includes the usage of chemical anticoccidial products (Mohsin et al. 2021a: 2021b). However, due to the emergence of drug resistance, the usage of these drugs against the disease is limiting (Abbas et al. 2019). Due to these reasons, usage of the botanicals and essential oils against coccidiosis is the best alternative (Abbas et al. 2012; 2017b).

Essential oils are rich in active compounds like phenols and sterols which possess therapeutic properties. Various *in-vitro* and *in-vivo* studies have proven many essential oils to have anticoccidial effects. They help controlling the disease either interfering with the parasitic metabolism directly or indirectly by enhancing the immune response of the host. As the *Eimeria* species produce oxidative stress, the antioxidant properties of essential oils prove an effective counter (Idris et al. 2017). *C. sinensis* essential oil has also been proved to have antioxidant effect (Jorge et al. 2016).

Keeping in view the importance of essential oils in controlling coccidiosis, the current *in-vitro* research was also aimed at investigation of anticoccidial potential of *C. sinensis* essential oil. The *in-vitro* experiment revealed *C. sinensis* essential oil to have anticoccidial effect. This essential oil was shown to exert a dose-dependent sporulation inhibition effect on the oocysts of mixed species of *Eimeria*. The bests results were exhibited at 10% concentration while the lowest sporulation inhibition was observed at 0.31% concentration. The results obtained for different concentrations of the *C. sinensis* essential oil were significant compared to the control groups at $P<0.05$ as shown in Fig. 1.

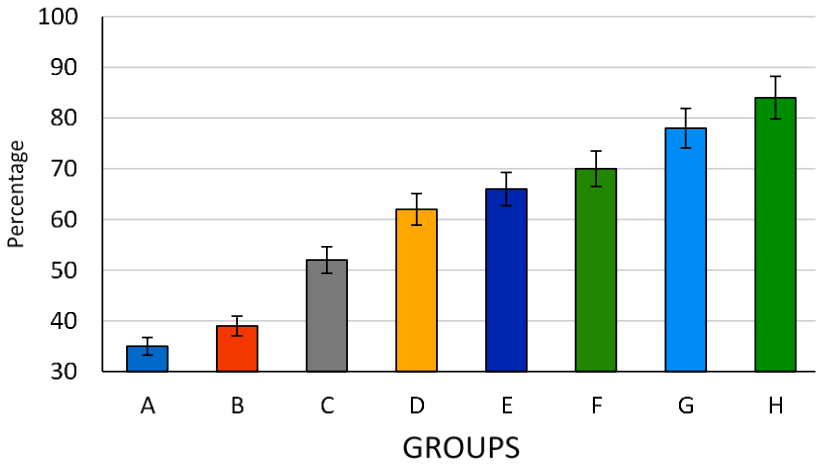


**Fig. 1:** Sporulation (%) of oocysts among different groups.

This anticoccidial effect may be attributed to the bioactive compounds present in it like limonene, linalool, myrcene and others (Sharma and Tripathi 2006). These compounds impart the anticoccidial effect to the *C. sinensis* essential oil. Limonene, the most abundant compound in *C. sinensis* essential oil, has been proven to have

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antioxidant and antiprotozoal effects (Arruda et al. 2009). Both these effects are helpful in controlling coccidiosis. Similarly, there are many other essential oils and their components with proven anticoccidial efficacy (Sidiropoulou et al. 2020; Langerudi et al. 2022).

## 4. CONCLUSION

It is concluded from the results of the current research that *C. sinensis* essential oil has anticoccidial potential. It exerts a dose-dependent inhibition effect on the sporulation of *Eimeria* oocysts. However, further research is needed to determine its *in-vivo* anticoccidial efficacy before being used in the coccidiosis control.

**Conflict of Interest**

Authors have no conflict of interest.

**Author's Contribution**

Aqsa Imran: Helped conducting experiment and data collection. Muhammad Salman: Writing original draft, editing, data analysis and methodology. Both authors approved final version of the manuscript.

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