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Original paper

Influence of essential oils on sporulation of *Eimeria magna* oocysts

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ABSTRACT. We 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* Perard, 1925. 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.* Thus, the study of the biological properties of essential oils of *C. verum* and *S. aromaticum* is of great interest for further research in the field of parasitology.

Keywords: *Eimeria magna*, *Cinnamomum verum*, *Syzygium aromaticum*, essential oils, oocysts, sporulation

Introduction

Aroma oily substances are mostly composed of cyclic hydrocarbons and their derivatives, and also alcohols, aldehides and esters. The main active oil constituents which have antiparasitic activity are phenylpropanoids (eugenol, methyl chavicol, cinnamaldehyde), sesquiterpenes (β-caryophyllene, nerolidol, α-copaene, cyperene, andgermacrene D), monoterpenoids (linalool, terpinen-4-ol, thymol, carvacrol, limonene, α-pinene, γ-terpinene, α-phellandrene and p-Cymene) and other aroma aldehides [1,2].

The mechanism of action of essential oils has not been studied completely. Each of the constituents of essential oil performs individual action towards various procariotic and eucariotic organisms [3–6]. The essential oils exert direct anticoccidial action by causing ultra-structural changes in the mitochondrial membrane and inhibition of glycoprotein syntheis and cysteine protease cruzain enzyme. Moreover, they have immunomodulatory effects, thus supporting the host and affecting parasites indirectly [7–10].

Currently, the effects of essential oils and their components on parasitic organisms are being actively studied. Some surveys report nematocidal properties of essential oils from *Cinnamomum verum* J. Presl (Lauraceae) and *Syzygium aromaticum* (L.) Merr. & L. M. Perry (Myrtaceae) during 24 h *in vitro* exposure causing death to 100% of nematode larvae of *Strongyloides papillosus* (Wedl, 1856) and *Haemonchus contortus* (Rudolphi, 1803) Cobb. Good results against larvae of *S. papillosus* were obtained using essential oil from *Melaleuca alternifolia* (Maiden & Betche) Cheel (Myrtaceae) [11].

Another relevant issue in the study of effects of essential oils on parasitic organisms is insecticidal properties. Many studies focused on influence of

essential oils on larvae of mosquitoes *Anopheles gambiae* Giles, 1902, *A. arabiensis* Patton, 1905 and *Culex quinquefasciatus* Say, 1823 [12–14]. Also, all around the globe, essential oils are surveyed for acaricidal properties: impacts of essential oils were reported for Acari *Psoroptes ovis* (Hering, 1838), *Sarcoptes scabiei* (Linnaeus, 1758), and also *Rhipicephalus microplus* (Canestrini, 1888) [15–17].

Coccidiosis of rabbits is a quite widespread parasitic disease around the world. Currently, there are many methods of combating *Eimeria* – pathogens of this disease [18–20]. However, the peculiarities of the life cycle of species of the *Eimeria* genus contribute to the rapid infection of new animals. The process of sporulation in *Eimeria* spp. occurs in the environment over 2–4 days depending on the environmental conditions, including temperature and air moisture. Such a short period before the invasive stage leads to constant repeated infestation of animals [21,22].

This parasite causes disorders in the intestine and the liver of rabbits: intestinal, hepatic and mixed forms are distinguished. At the beginning of the disease, the appetite deteriorates, then the intestine functions are impaired. Sometimes, spasms of limbs are recorded. Pathoanatomical changes indicate damage to the liver, urinary tract, and also the duodenum [23,24].

The objective of our article was evaluating the effect of the commonest essential oils on the vitality of *E. magna* oocysts in different stages of their development in laboratory conditions.

Materials and Methods

Sample collection

The research was conducted during 2020 at the Laboratory of the Department of Parasitology and Veterinary-Sanitary Expertise of Dnipro State Agrarian and Economic University (Ukraine). The samples of faeces were selected from rabbit of the Clinical-Diagnostical Center of the Faculty of the Veterinary Medicine of Dnipro Agrarian and Economic University which was naturally-infected with eimeriosis. The analysis of faeces for *E. magna* oocysts was conducted by the McMaster method, oocysts were identified by morphological characteristics [25,26].

Essential oils

For the experiment, we used 14 essential oils manufactured by Farmakom (Ukraine): *Piper cubeba* L.f., 1781, *Cananga odorata* (Lam.) Hook. F. & Thomson, *Pelargonium graveolens* L'Héritier, *Citrus sinensis* (Linnaeus) Osbeck (pro. sp.), *Eucalyptus globulus* Labillardière, *Lavandula angustifolia* Miller, *Picea abies* (L.) H. Karsten, *Citrus paradisi* Macfadyen, *Pterocarpus santalinus* L., *Abies sibirica* Ledeb., *Juniperus communis* Linnaeus, *Melaleuca alternifolia* (Maiden & Betche) Cheel, *Syzygium aromaticum* (L.) Merrill & Perry, *Cinnamomum verum* J. Presl.

Tree essential oils (*E. globulus*, *M. alternifolia*, *P. santalinus*) have dense, viscous aroma and have anti-inflammatory and antiseptic properties. On people they are used for treating colds and diseases of the respiratory tracts. Flower essential oils (*P. cubeba*, *C. odorata*, *L. angustifolia*, *P. graveolens*) have bactericidal, antiseptic, anti-inflammatory and other effects on the organisms of animals and humans. Coniferous oils (*P. abies*, *J. communis*, *A. sibirica*) have strong anti-inflammatory, antiseptic and antiviral effects. Aromatic essential oils (*S. aromaticum*, *C. verum*) are characterized by notable antiseptic, antibacterial and anti-inflammatory effects and less expressed toning and warming actions. Citrus aroma oils (*C. sinensis*, *C. paradisi*) stimulate skin regeneration, remove inflammation, and exert antiseptic effects [27].

In vitro assay

Oocysts of *E. magna* from the faecal samples were rinsed using normal saline and centrifuged over 2 min (1,500 rpm). In the laboratory conditions the mixture of unsporulated oocysts (on average 22.3 oocysts/sample) was submerged in 0.5% aqueous emulsion of essential oil in five replications, and also we studied the variant without addition of oil at 3 days exposure (28ºC). To prepare 0.5% emulsion, the essential oils were dissolved in normal saline. Afterwards, we examined the stage of sporulation in the control and the experimental emulsions under the microscope. We counted the amount of oocysts at different stages of sporulation: unsporulated, sporulated and partially sporulated oocysts.

Statistical analysis

The data was analyzed in Statistica 8 (StatSoft Inc., USA). In the diagrams the small squares show the median, the large rectangles show the 25% and 75% quartiles, the vertical lines show 95% of the variation, the stars and circles show the outliers. Data in the text were presented as mean ± standard

deviation (mean ± SD).

Results

We determined that 0.5% emulsion of six essential oils did not arrest the process of sporulation of *E. magna* (Fig. 1). 100 ± 1% and 94 ± 2% of the oocysts sporulated after 72-hour exposure to the oils from *P. cubeba* and *C. odorata* (Fig. 1B,C). At the same time, no delaying of the formation of sporocytes occurred. About 55–75% oocysts of *E. magna* sporulated under exposure to essential oils of *P. graveolens* (73 ± 8%, Fig. 1D), *C. sinensis* (63 ± 7%, Fig. 1E), *E. globulus* (64 ± 7%, Fig. 1F) and *L. angustifolia* (59 ± 5%, Fig. 1G). The development of the greatest quantity of oocysts was delayed by exposure to 0.5% emulsion of *L. angustifolia*. The development of small part of oocysts was delayed by essential oils from *P. graveolens* (9 ± 6%, Fig. 1D) and *E. globulus* (12 ± 3%, Fig. 1F). Emulsion of essential oil from *C. sinensis* affected the development of only 22 ± 7% of oocysts (Fig. 1E).

The process of sporulation was delayed by six

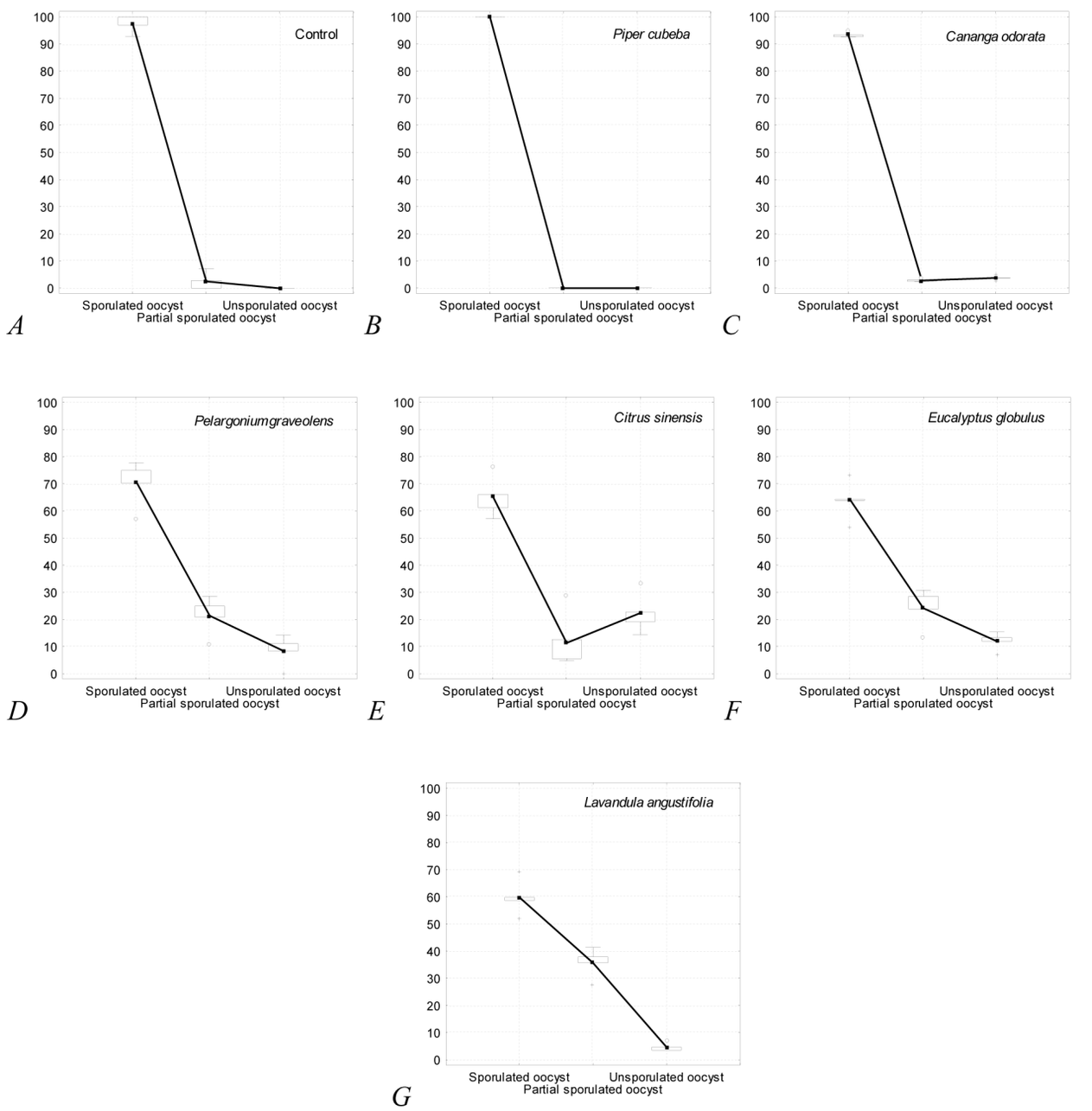

Figure 1. Essential oils of plants which had no or weak influence on sporulation of *E. magna* (n = 5):the ordinate shows the proportion (%) of the total number of oocysts in the experiment

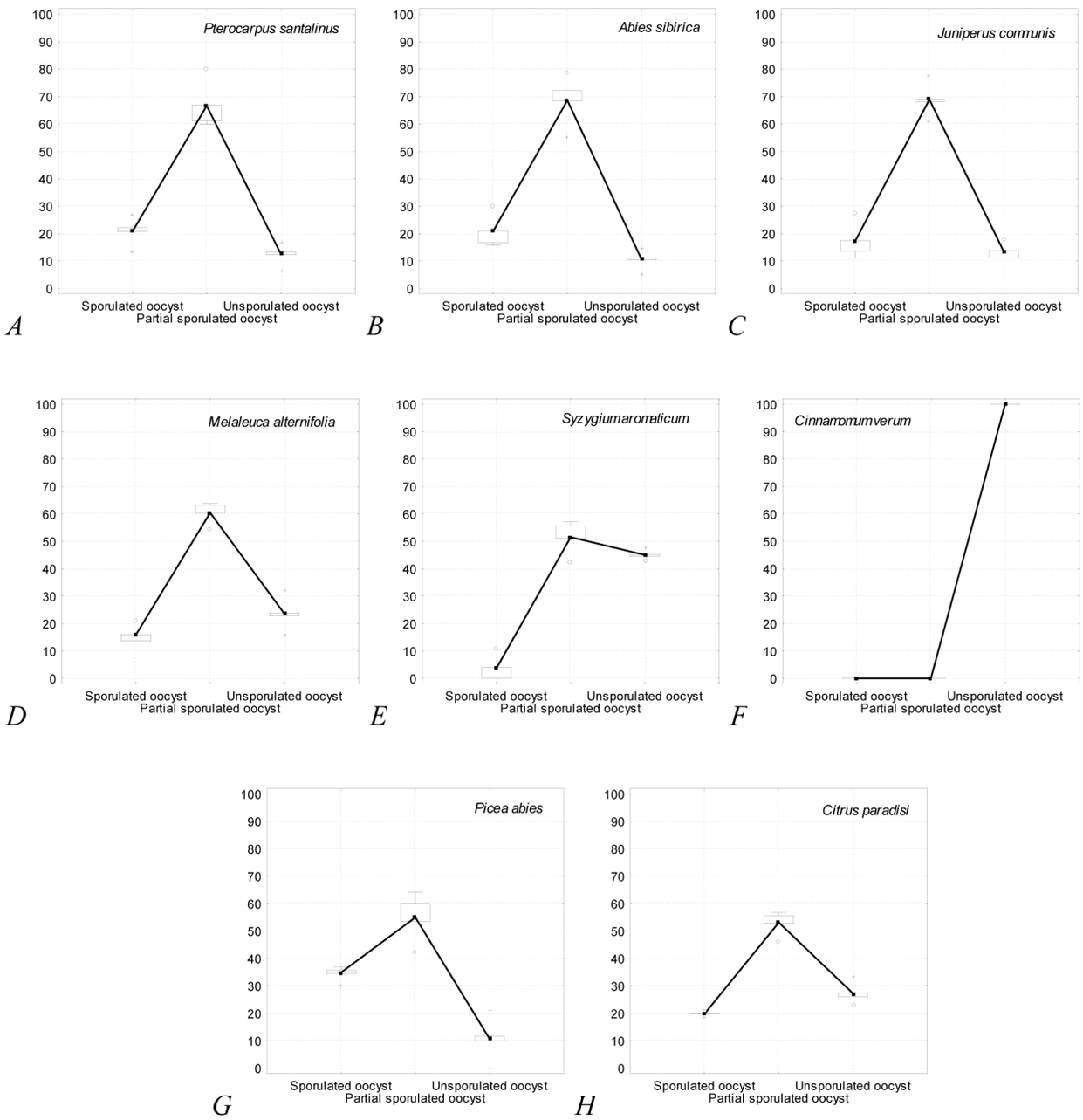

Figure 2. Plant essential oils which delayed or arrested the sporulation of *E. magna* (n = 5): the ordinate shows the proportion (%) of the total number of oocysts in the experiment

studied oils: *P. abies*, *C. paradisi*, *P. santalinus*, *A. sibirica*, *J. communis*, *M. alternifolia* (Fig. 2). Good results were seen while using essential oil from *S. aromaticum*: only 54% of oocysts were observed vital (3 ± 3%) and partially sporulated (51 ± 5%) after exposure to this essential oil (Fig. 2E). No sporulated oocysts at all were seen after 72 hours. The best results were produced by *C. verum,* 0.5% the emulsion of which killed 100% of *E. magna* oocysts during 72 hours (Fig. 2F).

Discussion

Thus, essential oils from *S. aromaticum* and *C. verum* were most efficient against *E. magna* oocysts. For more than 50% of oocysts, no sporulation was observed over exposure to 0.5% emulsions of these essential oils. In the literature, many works focus on the study of influence of essential oils and their constituents on the development on the oocysts of *Eimeria* spp. Remmal et al. [28] evaluated the ability of some main constituents of essential oils to impact on

oocysts of *Eimeria* spp. in *in vitro* conditions. According to these autors, carvacrol, carvone, isopulegol, thymol and eugenol are most active components against oocysts. Exposure of oocysts of *Eimeria* spp. to these constituents led to their lysis depending on the dose and time (0.3–2.0 mg/ml). Remmal et al. [28] determined that most effective essential oils were the ones from artemisia (*Artemisia absinthium*), tea tree (*Melaleuca alternifoli*), thymes (*Thymus vulgaris)* and clove (*Syzygium aromaticum*). These four essential oils destroyed oocysts of *Eimeria* over several hours at low concentration. Such effect is a consequence of their lysis. The data we obtained also indicate effectiveness of essential oil from clove against sporulation of oocysts. According to the results of our studies, 100% efficiency was exerted by 0.5% emulsions of oil from *C. verum*. In the experiment by Remmal et al. [28], this oil exerted destructive impact on oocysts (LC_{50} 3.917 mg/ml). In our experiment 0.5% emulsion of essential oil of paperbacks over 72 hours took no significant effect on sporulation of oocysts. At the same time, the membranes of oocysts remained undemaged. Differences in the results of studies were perhaps caused by duration of the experiment, manufacturer of oils in the conditions of germination of plants [29].

Many studies focused on the influence of essential oils on the course of coccidiosis in birds. Giannenas et al. [30] surveyed the effect of essential oil from *Origanum vulgare* L. (Lamiaceae) on productivity of broiler chickens, experimentally infected by *Eimeria tenella* (Tyzzer, 1929) at the age of 14 days, and determined that the essential oil had anti-coccidiosis effect. However, this effect was lower than during the use of lasalocid. Upadhaya et al. [31] indicate that mixtures of essential oils and vitamin D_3 also could be used as anti-coccidiosis food additive in coccidiosis infection of broilers.

Sorour et al. [32] surveyed anticoccidial and hepatoprotective effects cinnamon essential oil and clove essential oil against *Eimeria stiedae* (Lindemann, 1865), parasites of rabbits. They determined that essential oils from cinnamon and carnation have protective properties against coccidiosis of the liver. Kowalska et al. [33] also studied the interaction of food additives based on the natural oils of oregano and garlic added to adequate diets as natural alternatives to coccidiostats in rabbit nutrition. They presumed that the extracts of herbs could be efficiently used for preventing infections as a natural alternative of coccidiostatics in feeds. Anti-coccidiosis properties of *Origanum* were also studied by Grandi et al. [34], though in their *in vivo* experiments they observed no decrease in the number of oocysts of *Eimeria* in faeces of cattle. Therefore, the results of the studies mentioned above, as well as our experiments concerning anticoccidial properties of essential oils, indicate the necessity of studies in this filed regarding essential oils from other species of plant in order to find alternatives for treating coccidiosis.

Out of essential oils from 14 species of plants we studied (*P. cubeba*, *C. odorata*, *P. graveolens*, *C. sinensis*, *E. globulus*, *L. angustifolia*, *P. abies*, *C. paradisi*, *P. santalinus*, *A. sibirica*, *J. communis*, *M. alternifolia*, *S. aromaticum*, *C. verum*) concerning their effect on sporulation of *E. magna*. 0.5% emulsion of essential oil from *C. verum* displayed powerful action towards oocyte parasites: 100% of unsporulated oocysts died over 72 hours. Many of the essential oils we studied can delay the sporulation of *Eimeria*: *P. abies*, *C. paradisi*, *P. santalinus*, *A. sibirica*, *J. communis*, *M. alternifolia*. Oils of *P. cubeba*, *C. odorata*, *P. graveolens*, *C. sinensis*, *E. globulus*, *L. angustifolia* took no effect of sporulation of *E. magna*.

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