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Nematicidal activity of essential oils of medicinal plants

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Abstract

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We studied the effect of essential oils from *Picea abies* (Linnaeus) H. Karsten., 1881, *Cinnamomum verum* J. Presl, 1825, *Melaleuca alternifolia* (Maiden & Betche) Cheel, 1925, *Citrus paradisi* Macfadyen, 1830, *Rosmarinus officinalis* Linnaeus, 1753, *Citrus aurantiifolia* (Christmann) Swingle, 1913, *Syzygium aromaticum* (L.) Merrill & Perry, 1939, *Pterocarpus santalinus* Linnaeus filius, 1782, *Pelargonium graveolens* L'Héritier, 1789, *Eucalyptus globulus* Labillardière, 1861, *Juniperus communis* Linnaeus, 1753, *Piper cubeba* L.f., 1781, *Calendula officinalis* L., 1753, *Laurus nobilis* Linnaeus, 1753, *Lavandula angustifolia* Miller, 1768 and *Citrus sinensis* (Linnaeus) Osbeck (pro. sp.) on vitality of free-living larvae of *Strongyloides papillosus* (Wedl, 1856) and *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898, and also survivability of eggs of *Ascaris suum* (Goeze, 1782) under *in vitro* conditions. The most notable nematicidal properties belong to 0.5% water emulsion of essential oils from *C. verum* and *S. aromaticum*: we observed 100% mortality of larvae of *S. papillosus* $L_{1–3}$ and *H. contortus* L_3.

Keywords

eggs, essential oils, flavourings, larvae, nematodes, mortality

Introduction

Among helminths of cattle, as well as wild ungulates, quite often recorded species are *Strongyloides papillosus* (Wedl, 1856) and representatives of *Strongylata* order. In swine breeding complexes and wild hogs in natural ecosystems, *Ascaris suum* (Goeze, 1782) dominates. Diseases they cause lead to big economic losses for farmers. Global need for the transition to non-chemical (organic) agriculture has given an impulse to reducing the use of veterinary preparations of synthetic origin and developing new plant-based preparations. All this has stimulated the use of natural and food additives which are identical to natural ones with anthelmintic, insecticidal, acaricidal, and antiprotozoal properties. Part of this research is focused on the effect of aqueous and alcohol extracts of medical plants on vitality of parasites in the environment (Boyko and Brygadyrenko, 2016, 2018, 2019a, 2019b; Zazharska et al., 2018; Palchykov et al., 2019).

Currently, data on use of essential oils from medicinal plants against different stages of parasites and agricultural pests are appearing with increasing frequency. These oils are applied in different concentrations, both *in vitro* and *in vivo* (Kweka et al., 2011, 2012; Rey-Valeirón et al., 2017; Chen et al., 2019; Martynov et al., 2019a, 2019b; Zhou et al., 2019). Most often research focuses on mortality of parasites after exposing them to solutions of essential oils with exposure ranging 24–72 h. Lethal concentrations of essential oils, as well as exposure to their effective action against insects and Acari pests of agriculture and ectoparasites have been studied. The objective of this study was the determination of vitality of nematode larvae

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of ruminants, and also eggs of nematodes of swine under the influence of essential oils from medicinal plants.

Materials and methods

The studies were performed at the Department of Parasitology and Veterinary-Sanitary Expertise of the Dnipro State Agro-Economic University. In the experiment, we used first- to third-stage larvae of the nematode of ruminants *S. papillosus* (rhabditiform first and second non-infective stages, filariform infective third stage), *H. contortus* at the third stage (infective), and also immature (without a developed larva) eggs of swine nematode *A. suum*. Larvae and eggs were identified by morphological features (Van Wyk et al., 2004, 2013; Zajac et al., 2011). Parasitological surveys for detecting eggs of nematodes were performed using the McMaster method. Identification of larvae was made by cultivation in a thermostat at the temperature of 24 °C over 8 days. For this purpose, the excrement of small ruminants (by 10 g) was put into Petri dishes, which were then put into the thermostat. The samples in the thermostat were moistened every 24 h. Larvae of *S. papillosus* at different stages were observed after 24–72 h, *H. contortus* at the third stage – after 8 days. The surveys of excrement for presence of nematodes were made using the Bermann method.

After cultivation of the biomaterial, the larvae in normal saline (4 mL) in test tubes (10 mL) were centrifuged at 1,500 rtp over 4 min. The sediment with larvae was by 0.1 mL (average 70–110 larvae) put into plastic test tubes of 1.5 mL capacity. Then, to the weighed amount with larvae 0.5% emulsion solutions of essential oils (in normal saline) of *Picea abies* (Linnaeus) H. Karsten., 1881 (limonene, bornyl acetate, δ-cadinene, α-muurolol, α-cadinol and other), *Cinnamomum verum* J. Presl, 1825 (α-copaene, α-bergamotene, α-humulene, δ-cadinene), *Melaleuca alternifolia* (Maiden & Betche) Cheel, 1925 (α-terpinene, γ-terpinene, terpinen-4-ol), *Citrus paradisi* Macfadyen, 1830 (limonene), *Rosmarinus officinalis* Linnaeus, 1753 (α-pinene, linalool, piperitone), *Citrus aurantiifolia* (Christmann) Swingle, 1913 (1-methoxycyclohexene, corylone, 3-methyl-1,2-cyclopentanedione, 5,7-dimethoxycoumarin), *Syzygium aromaticum* (L.) Merrill & Perry, 1939 (eugenol, eugenol acetate), *Pterocarpus santalinus,* Linnaeus filius, 1782 (*cis*-α-santalol, *cis*-β-santalol), *Pelargonium graveolens* L'Héritier, 1789 (β-citronellol, geraniol, citronellyl formate, 10-epi-γ-eudesmol), *Eucalyptus globulus* Labillardière, 1861 (1,8-cineole, α-pinene), *Juniperus communis* Linnaeus, 1753 (α-pinene, sabinene), *Piper cubeba* L.f., 1781 (citral, geraniol, citronellal), *Calendula officinalis* L., 1753 (flavonoids and carotenoids), *Laurus nobilis* L., 1753 (cineole, (-)-linalool, myrcene, (+)-limonene, α- and β-fellandrenes), *Lavandula angustifolia* Miller, 1768 (linalool, myrcene, α- and β-otsimen, γ-terpinene, α-pinene, karyofillen, bergamoten) and *Citrus sinensis* (Linnaeus) Osbeck (pro. sp.) (limonene and other) were added. All experiments were performed in seven replications (Khejfits et al., 1994; Vojtkevich, 1999; Sellar, 2005). As the control, 0.9% solution of NaCl was used. "Farmakom" (Ukraine) – manufacturer of essential oils. Then the samples were put into the thermostat for 24 h. For proving death of the larvae, we took into account two factors: immobility and decomposition of intestinal cells.

Eggs of *A. suum* were washed; for this purpose, the feces of 10 g mass were put into a glass cup and 100 mL of water was added. The eggs were washed several times until a clean sediment was obtained. The water-washed eggs were put into plastic test tubes (1.5 mL) by 0.1 mL in each (average 5–6 eggs). Then, 1 mL of emulsion solutions of essential oils were added, and also normal saline (in the control) in seven replications. The processing of the immature eggs with essential oils in 0.5% concentrations was made during 24 h. Then the eggs of *A. suum* were washed with distilled water. The test tubes were put into the thermostat. The eggs were cultivated for 21 days at the temperature of 28 °C. Then, presense or absence of developed larvae was determined.

The statistical analysis of the results was performed through a set of Statistica 8.0 (StatSoft Inc., USA). The data in the Table are given in the form of mean ± standard deviation (x ± SD). Reliability of differences between the control values and plant preparations are given according to ANOVA.

Results and discussion

For the most types of essential oils the mortality of non-invasive larvae *S. papillosus* L_{1-2} was significantly higher compared with infective stages of *S. papillosus* L_3 and *H. contortus* L_3 (Table 1). Exposure of the larvae to 0.5% essential oils from *P. abies*, *L. nobilis*, *P. santalinus*, *P. graveolens*, *E. globulus*, *C. paradisi*, *C. sinensis*, *C. officinalis* and *R. officinalis* for 24 h causes the death of *S. papillosus* and *H. contortus* larvae and eggs of *A. suum.*

A stronger effect was caused by 0.5% emulsion solution of essential oil of *C. aurantiifolia*. Around 8% of the third-stage larvae of *S. papillosus* were killed after 24 h exposure to this substance. Most susceptible were the first-second stage *S. papillosus* larvae: essential oil of lime in 0.5% decimated around 82% of larvae of these stages. The infective larvae of *H. contortus* were the most resistant to the essential oil of lime, no dead larvae were observed. Essential oil from *M. alternifolia* also exhibited no effect on third-stage larvae of *H. contortus*. However, a stronger effect was shown by *M. alternifolia* against the larvae of *S. papillosus* at different stages: first and second stage larvae died in 95% of cases, third-stage larvae – in 69%.

The essential oil from *C. verum* was the most efficient against the nematode larvae. Larvae of *S. papillosus* of different stages, as well as third-stage *H. contortus* died in 100% of cases during 24 h exposure to 0.5% emulsion solution of cinnamon essential oil.

Oil from *S. aromaticum* also exerted a negative effect on different-stage nematode larvae: exposure of *S. papillosus* L_{1-3} and *H. contortus* to 0.5% emulsion solution of this oil resulted in killing all the larvae.

Table 1. Mortality of larvae and eggs of parasitic nematodes (%) during 24 h laboratory experiment (x ± SD, n = 7) exposed to essential oils

Plant family	Plant species	Nematode species and stage of development	Mortality of nematode larvae in plant oil solution, %	Mortality of nematode larvae in control, %	P
Pinaceae	*Picea abies* (L.) H. Karsten., 1881	*S. papillosus*, $L_{1–2}$	11.5 ± 4.7	8.4 ± 3.0	–
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Cupressaceae	*Juniperus communis* Linnaeus, 1753	*S. papillosus*, $L_{1–2}$	12.9 ± 4.5	12.1 ± 2.4	–
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Lauraceae	*Cinnamomum verum* J. Presl, 1825	*S. papillosus*, $L_{1–2}$	100.0 ± 0.0	8.4 ± 3.0	< 0.001
		S. papillosus, L_3	100.0 ± 0.0	0.0 ± 0.0	< 0.001
		H. contortus, L_3	100.0 ± 0.0	0.0 ± 0.0	< 0.001
		A. suum, egg	74.3 ± 12.5	0.0 ± 0.0	< 0.001
Lauraceae	*Laurus nobilis* L., 1753	*S. papillosus*, $L_{1–2}$	13.2 ± 2.5	12.1 ± 2.4	–
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Piperaceae	*Piper cubeba* L.f., 1781	*S. papillosus*, $L_{1–2}$	77.3 ± 3.5	12.1 ± 2.4	< 0.001
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Fabaceae	*Pterocarpus santalinus* Linnaeus filius, 1782	*S. papillosus*, $L_{1–2}$	14.2 ± 2.5	12.1 ± 2.4	–
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Geraniaceae	*Pelargonium graveolens* L'Héritier, 1789	*S. papillosus*, $L_{1–2}$	10.6 ± 2.8	12.1 ± 2.4	–
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Myrtaceae	*Melaleuca alternifolia* (Maiden & Betche) Cheel, 1925	*S. papillosus*, $L_{1–2}$	95.4 ± 1.9	8.4 ± 3.0	<0.001
		S. papillosus, L_3	68.8 ± 14.7	0.0 ± 0.0	<0.001
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Myrtaceae	*Syzygium aromaticum* (L.) Merrill & Perry, 1939	*S. papillosus*, $L_{1–2}$	100.0 ± 0.0	8.6 ± 2.3	<0.001
		S. papillosus, L_3	100.0 ± 0.0	0.0 ± 0.0	<0.001
		H. contortus, L_3	100.0 ± 0.0	0.0 ± 0.0	<0.001
		A. suum, egg	65.0 ± 22.6	0.0 ± 0.0	<0.001
Myrtaceae	*Eucalyptus globulus* Labillardière, 1861	*S. papillosus*, $L_{1–2}$	10.2 ± 2.6	12.1 ± 2.4	–
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Rutaceae	*Citrus aurantiifolia* (Christmann) Swingle, 1913	*S. papillosus*, $L_{1–2}$	82.4 ± 4.0	8.4 ± 1.2	<0.001
		S. papillosus, L_3	7.7 ± 6.7	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–

Table 1. Continued

Rutaceae	*Citrus paradisi* Macfadyen, 1830	*S. papillosus*, $L_{1–2}$	11.0 ± 0.9	10.6 ± 2.7	–
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Rutaceae	*Citrus sinensis* (L.) Osbeck (pro. sp.)	*S. papillosus*, $L_{1–2}$	9.4 ± 2.2	12.1 ± 2.4	–
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Asteraceae	*Calendula officinalis* L., 1753	*S. papillosus*, $L_{1–2}$	9.8 ± 1.7	12.1 ± 2.4	–
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Lamiaceae	*Lavandula angustifolia* Miller, 1768	*S. papillosus*, $L_{1–2}$	50.0 ± 4.4	12.1 ± 2.4	<0.001
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–
Lamiaceae	*Rosmarinus officinalis* Linnaeus, 1753	*S. papillosus*, $L_{1–2}$	9.4 ± 0.9	8.4 ± 1.2	–
		S. papillosus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		H. contortus, L_3	0.0 ± 0.0	0.0 ± 0.0	–
		A. suum, egg	0.0 ± 0.0	0.0 ± 0.0	–

"–", effect of essential oil from plant on vitality is absent; < 0.001, reliability of differences between the control values and plant preparations according to ANOVA.

Use of the oil of *L. angustifolia* caused death to 50% of first- and second-stage larvae of *S. papillosus*. All the infective larvae were resistant to the effect of this plant preparation. Similar results were recorded for use of essential oil from *P. cubeba*: around 77% of non-infective larvae survived after 24 h exposure to emulsion solution of this essential oil.

Studies of the effect of essential oils on vitality of eggs of *A. suum* revealed the effect of two preparations: *C. verum* and *S. aromaticum*. Over 24 h exposure of immature eggs to 0.5% emulsion solution of essential oil from *C. verum,* we observed death of around 74% of eggs. Exposure to essential oil from *S. aromaticum* caused death to 65% of eggs of *A. suum*. Emulsion solutions of essential oils of the rest of the studied plants (Table 1) demonstrated no reliable effect on the development of eggs of *A. suum*.

Study of the level of the effect of essential oil on the vitality of parasites, including eggs and larvae of nematodes *in vitro* is relevant today. In the literature one can find data on the effect of essential oils from fruits and seeds of *Schinus terebinthifolia* Raddi on the vitality of larvae of mosquitoes *Anopheles gambiae* Giles, 1902, *A. arabiensis* Patton, 1905 and *Culex quinquefasciatus* Say, 1823, vectors of malaria and many dangerous viral diseases, in the environment (Kweka et al., 2011). Kweka et al. (2011) propose alternative insecticides with the use of this essential oil. Similar experiments were also undertaken by Kweka et al. (2012).

The essential oil from *Cinnamomum osmophloeum* Kaneh was used against larvae of the mosquito *A. gambiae*. In spite of the growing resistance to insecticides of mosquitoes in the African countries, Mdoe et al. (2014) investigated the essential oil of *C. osmophloeum* in order to find an alternative compound against the vectors of malaria.

The main components of the studied essential oil from *C. verum* are α-bergamotene (27.4%) and α-copaene (23.1%); in a lower amount it contains α-humulene (6.2%), δ-cadinene (6.0%), tetradecanol (4.3%), viridiflorene (3.3%), α-muurolene (2.7%), trans-cinnamyl acetate (2.4%), germacrene D (2.1%), epi-α-bisabolol (2.1%), spathulenol (2.0%) and other components (Jayaprakasha et al., 2002). Further studies on nematocidial activity of these components would be promising.

Silva et al. (2010) also studied altenative methods of control of mosquitoes of the genus *Aedes* and its allies, such as *Stegomyia*, which transmit diseases such as dengue and yellow fever, by using essential oil of Brazilian pepper. Insecticidal properties of essential oils against parasites and pests of agriculture were also surveyed in the works by Pavela (2006), Boutoumi et al. (2009), Samarasekera et al. (2006), Clemente et al. (2008), Zoubiri et al. (2012), Lamari et al. (2014), Essolakina et al. (2014), Parsia et al. (2016), Kolani et al. (2016), Khoobdel et al. (2017), Hennia et al. (2019). No less relevant are the studies on the effect of essential oils on the vitality of Acari in the environment. Chen et al. (2019) described the mortality level of *Psoroptes ovis* (Hering, 1838), mites, sheep parasites, exposed to four components of essential oil of plant origin (geraniol, eugenol, 1,8-cineole and carvacrol).

Against Acari *Sarcoptes scabiei* (Linnaeus, 1758), parasites of agricultural animals, similar *in vitro* studies were undertaken by Zhou et al. (2019). They indicate acaricidal effect of essential oil from *Elsholtzia densa* Benth. Against *Tetranychus urticae* Koch mites (Acari, Tetranychidae), agricultural pests of vegetables and decorative plants, Çalmaşur et al. (2006) used vapours of essential oils from *Micromeria fruticosa* L., *Nepeta racemosa* L. and *Origanum vulgare* L. (Lamiaceae). Similar studies were performed by Neves et al. (2011), Attia et al. (2012), Fatemikia et al. (2017), and also Da Camara et al. (2017). Rey-Valeirón et al. (2017) have proved the acaricidal effect of essential oils from *Bursera graveolens* (Kunth) Triana & Planch. (Burseraceae) and *Schinus molle* Linnaeus, 1753 (Anacardiaceae).

Currently, the influence of essential oils on helminths of domestic animals and humans is being actively studied. Singh et al. (2009) studied the inhibitory effect of essential oils of *Allium sativum* L. and *Piper longum* L., Jeyathilakan et al. (2010) – of *Cymbopogon nardus* L. (citronella) and *Azadirachta indica* A. Juss. (Neem) on *Fasciola gigantica* Cobbold, 1858. Anthelmintic effect of *Mentha* spp. essential oils on *Echinococcus granulosus* (Batsch, 1786) is described in the study by Maggiore et al. (2011). There are studies on the effect of essential oils on vitality of nematodes. Therefore, Olounladé et al. (2011) studied the in vitro anthelmintic activity of the essential oils of *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler and *Newbouldia laevis* Seem. against *Strongyloides ratti* Sandground, 1925. Katiki et al. (2011) studied the anthelmintic activity of essential oils from *Cymbopogon martini* (Roxb.) Wats., *C. schoenanthus* Spreng. and *Mentha piperita* L. The experiments were performed on the eggs and larvae of nematodes *H. contortus* and *Trichostrogylus* spp. They revealed that essential oil from *C. schoenanthus* could be used against nematodes. Similar experiments were undertaken by Saha and Lachance (2019) and Macedo et al. (2019). Their results suggest that EOs from the genus *Cymbopogon* can be interesting candidates for nematode control in cattle.

The results of all these studies partly coinside with our experiments regarding the effect of a variety of essential oils against parasitic organisms, including larvae of *H. contortus* and *S. papillosus*, and eggs of *A. suum*.

Conclusion

In vitro conditions revealed perspectives for further use of essential oils of *Cinnamomum verum*, *Piper cubeba*, *Melaleuca alternifolia*, *Syzygium aromaticum*, *Citrus aurantiifolia*, and *Lavandula angustifolia* against parasitic nematodes of agricultural animals. The most notable nematocidial properties were exerted by the emulsion solutions of essential oils from *Cinnamomum verum* and *Syzygium aromaticum*. Exposure of different stages of nematode larvae of ruminants (*S. papillosus* L_{1-3} and *H. contortus* L_3) to these oils was followed by the death of 100% of them. The results of the experiments can be used for the development of complex methods of protecting agricultural anaimals against the commonest species of nematods.

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