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Sustainability of pasture ecosystems: The use of plant-based mixtures in the fight against helminths

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In developing countries, cattle grazing is associated with damage from contaminated manure as one of the hazardous types of organic waste. This, in turn, threatens the sustainability of various livestock sectors and causes significant economic losses. Such countries need to develop new sustainable strategies for integrated control of animal helminthiasis. Parasitic nematodes can significantly reduce the productivity and viability of agriculturally important mammals and are very common in human populations where sanitary standards are not met. In recent years, there has been a significant trend of parasite resistance to chemically synthesized veterinary treatment, including broad-spectrum anthelmintics. The chemicals used with anthelmintic properties pollute pasture ecosystems. In order to achieve environmentally sustainable development and minimize the impact of these substances on the environment we determined in a laboratory experiment investigating the effects of eugenol, isoeugenol, thymol and carvacrol, individually and in paired mixtures, on *Strongyloides papillosus* and *Haemonchus contortus* larvae and *Trichiuris vulpis* and *Toxocara canis* eggs. Mortality of *S. papillosus* larvae exposed to a 0.01% mixture of isoeugenol and thymol (97.5 ± 1.7%), eugenol and thymol (96.6 ± 2.6%), and thymol and carvacrol (92.0 ± 5.8%) exceeded 90%. Under the influence of a mixture of 0.01% eugenol and isoeugenol (89.1 ± 10.3%), eugenol and carvacrol (88.2 ± 8.5%), mortality was insignificantly reduced. *Haemonchus contortus* larvae were significantly more resistant to the influence of mixtures of 0.01% of the studied substances: maximum mortality was noted for eugenol and isoeugenol (80.0 ± 10.6%), eugenol and thymol (86.8 ± 9.6%). Under the influence of 0.1% of the studied substances applied separately, mortality of *T. vulpis* eggs reached its maximum for carvacrol (80.2 ± 11.4%), was significantly lower for thymol (55.3 ± 7.5%) and remained at the level of the control group (without influence of either of the substances) for eugenol and isoeugenol. Average mortality of *T. vulpis* eggs exceeded 90% when exposed to 1.0% thymol, eugenol, and isoeugenol. At this dosage, carvacrol inflicted the lowest mortality of the four substances studied for *T. vulpis* eggs. Under the influence of mixtures of these substances for 24 hours, larvae subsequently formed in the eggs of *Trichiuris vulpis* and *Toxocara canis* in more than 68% of cases. Mortality of eggs of *T. vulpis* was highest when exposed to a mixture of isoeugenol and carvacrol. Among the studied nematode species, the eggs of *T. canis* turned out to be the most resistant to the mixtures of the studied substances: the mortality of eggs did not exceed 16%. Our research results show that mixtures of even very low concentrations of eugenol with thymol can reduce the viability of larvae of nematode species that are important in medicine and veterinary medicine.

Keywords: sustainable agriculture; minimizing environmental impact; mortality of nematodes; Lamiaceae; Myrtaceae; *Strongyloides papillosus*; *Haemonchus contortus*; *Trichiuris vulpis*; *Toxocara canis*.

Introduction

The rapid development of the economy and agriculture is accompanied by a constant increase in organic waste (manure, wastewater, solid municipal waste, etc.). These wastes contain many organic substances, and also contain various types of toxins, pathogens and chemically synthesized drugs, including antibiotics and anthelmintics (Wang et al., 2018). Environmentally friendly alternative measures for helminth control include genetic resistance of host organisms, vaccines and biological control. These measures are promising and important for the sustainability of pasture ecosystems. However, they require further search for non-toxic substances with anthelmintic properties (Donald, 1994; Morgan et al., 2013). Eugenol, isoeugenol, thymol, and carvacrol are organic substances that are contained in the essential oils of many plants from the Lamiaceae, Myrtaceae, Apiaceae families, including medicinal ones.

Thymol is a component of the essential oils of *Thymus vulgaris* L. (Lamiaceae) (Salehi et al., 2018; Kowalczyk et al., 2020), *Trachyspermum ammi* (L.) Sprague (Apiaceae) (Biswal et al., 2021), as well as essential oils of plants of the genera *Ocimum*, *Origanum*, *Monarda* (Lamiaceae) and some representatives of the families Verbenaceae, Scrophulariaceae, Ranunculaceae and Apiaceae (Marchese et al., 2016; Barbosa et al., 2020). The main component of *Plectranthus amboinicus* Lour. (Lamiaceae) is carvacrol (Resende et al., 2022; Thanh et al., 2024). It is also a component of plants of the *Origanum* genus, including *Origanum vulgare* L. (Lamiaceae) and many species of the *Thymus* genus (Lamiaceae) (Mondal et al., 2020; Rathod et al., 2021). Isoeugenol and eugenol are components of the oil of *Syzygium aromaticum* (L.) Merr. & L. M. Perry (Myrtaceae) (Xie et al., 2015; Topal, 2019).

Many facts presented in our previous studies (Boyko & Brygadyrenko, 2019a; Boyko et al., 2020) testify to the antiparasitic (including anthelmintic) properties of plants. Some essential oils with the substances under study also have nematicidal and protistocidal properties. (Boyko et al., 2021; Boyko & Brygadyrenko, 2021).

The search for new environmentally safe substances with anthelmintic properties will reduce the toxic load on natural ecosystems and agrocenoses for agricultural waste management and help to achieve the sustainability of pasture ecosystems (Agrawal et al., 2024). Therefore, the aim of our study is to evaluate the effects of mixtures of eugenol, isoeugenol, thymol, and carvacrol *in vitro* on the viability of eggs and larvae of nematodes, which are dangerous parasites of farm and carnivorous animals, as well as humans.

Materials and methods

Study area. The site of excrement sampling was located in central Ukraine – Dnipro Region (48.421433° N, 35.051520° E). This part of

Ukraine is located in the temperate latitudes. The most characteristic features of its climate are continentality and dryness. The coefficient of humidity is within 0.55–0.65. The steppe climate is characterized by significant daily fluctuations of the temperature and uneven precipitation throughout the year. The mean annual air temperature is +9.5 °C. In July and August the mean temperature is 25.3 and 25.1 °C, in January and February - –1.4 and –3.3 °C. Most precipitation occurs in May and June (72 and 70 mm). 25–44 mm of precipitation falls in other months. The least amount of precipitation falls in October and November (30 and 25 mm). In summer, there are quite frequent showers, while overall 20% of annual precipitation falls as snow. On average, 565.2 mm of precipitation falls in this area per year. The number of days with precipitation in the region is between 120 and 160 (https://weatherandclimate.com/ukraine/dnipropetrovsk/dnipro, https://en.wikipedia.org/wiki/Dnipro).

Sampling. Samples of excrement were collected in Dnipro Region from naturally infected animals (small ruminants, dogs) of the educational, scientific, production, clinical and diagnostic center of the Faculty of Veterinary Medicine of the Dnipro State Agrarian and Economic University.

20 g of each sample was placed in laboratory boxes and transported to the parasitological research laboratory of Parasitology and Veterinary Expertise Department of the Faculty of Veterinary Medicine of the Dnipro State Agrarian and Economic University. The samples were stored in a refrigerator at a temperature of +4 °C before the start of the research.

Experimental design. There were two stages of the experiment. At the first stage of the experiment, the effect of mixtures of previously studied nematicidal properties of substances (thymol, carvacrol, eugenol, isoeugenol) on nematode larvae was studied (Boyko & Brygadyrenko, 2023). The second stage of the experiment was devoted to the effect of these substances and their mixtures on the survival of nematode eggs, which were most resistant to environmental factors (Boyko & Brygadyrenko, 2020).

Biological material for research. Larvae (Boyko & Brygadyrenko, 2018) and eggs of animal nematodes (Boyko & Brygadyrenko, 2017) were used as biological models for research (Fig. 1): first and second age larvae ($L_{1–2}$) of *Strongyloides papillosus* (Wedl, 1856) Ransom, 1911 (Rhabditida, Strongyloididae) and third age larvae (L_3) of *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898 (Rhabditida, Trichostrongylidae) (parasites of ruminants), as well as immature eggs of carnivorous parasites – *Trichuris vulpis* (Froelich, 1789) Smith, 1908 (Trichinellida, Trichuridae) and *Toxocara canis* (Werner, 1782) Stiles, 1905 (Rhabditida, Ascarididae).

The feces were examined for the presence of nematode eggs using the generally accepted McMaster method (Zajac, 2011).

The larvae of the nematodes *S. papillosus* and *H. contortus* were identified by morphological features (Van Wyk et al., 2004; Van Wyk & Mayhew, 2013). The body size, shape of the tail end, and internal structure were taken into account, namely: *H. contortus* has 16 triangular intestinal cells located in two rows and the intestine of *S. papillosus* has the form of two dark strands. The presence of an esophagus with bulb-like formations in the esophagus (one or two) was also taken into account in the $L_{1–2}$ *S. papillosus*.

Trichuris vulpis eggs were identified by size (70–90 x 32–41 μm), shape (lemon-shaped), colour (brown), shell shape (thick with smooth surface), presence of transparent polar plugs and absence of formed larvae (Thienpont et al., 2003; Zajac, 2011).

Toxocara canis eggs were also identified by size (75 x 85–90 μm), shape (nearly spherical, sometimes oval), colour (dark-brown), shell shape (thick, pitted). Eggs of this nematode species also do not have larvae (Thienpont et al., 2003; Zajac, 2011).

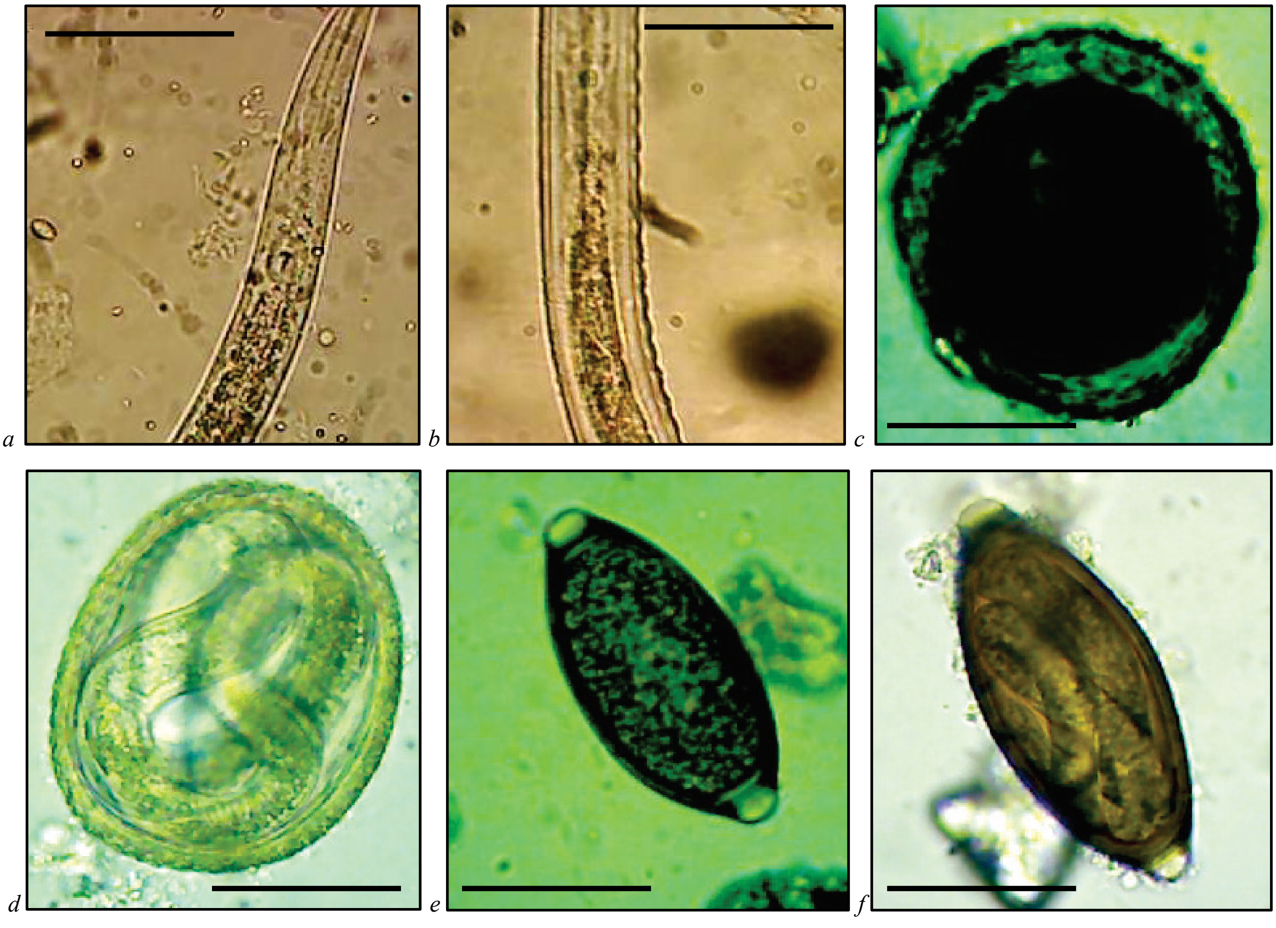
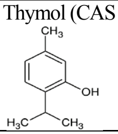
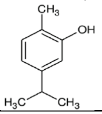
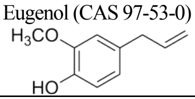
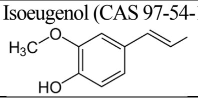


Fig. 1. *Strongyloides papillosus* (*a*) and *Haemonchus contortus* (*b*) larvae: the head end of the body with elements of the esophagus and intestine, the white pointer indicates the expansion of the esophagus in rhabditiform larvae, the black one – to the intestinal cells; *Toxocara canis* eggs: immature (*c*) and mature, with a formed larva (*d*); *Trichiuris vulpis* eggs: immature (*e*) and mature, with a formed larva (*f*); length of black bar – 50 μm

Cultivation of nematode larvae. Cultivation of ruminant nematode larvae *S. papillosus* and *H. contortus* was carried out according to their life cycle in the environment for 3 and 10 days, respectively (Zajac, 2011). For this purpose, feces from small ruminants were placed in 10 mL test tubes at a temperature of 22 °C and moistened every 24 hours with 1 mL of room temperature water (18–22 °C). A TC-20 medical dry-air thermostat (Micromed, Kharkov, Ukraine, 2023) was used to maintain a constant temperature. The test tubes with feces were covered with cotton balls to reduce moisture evaporation. Nematode larvae (Fig. 1a, 1b) were identified using the Baermann method, followed by centrifugation of the sediment with larvae. The larval culture was centrifuged for 2 minutes at 1500 rpm using a Micromed CM-3M centrifuge (Micromed, Kharkov, Ukraine, 2022).

Preparation of solutions. For the experiment, solutions of thymol, carvacrol, eugenol, isoeugenol (Table 1) in concentrations of 1% and 0.1% were preliminarily used (Boyko & Brygadyrenko, 2022). In order to determine the synergistic effect of the studied substances, the following mixtures were used: eugenol (0.01%) + carvacrol (0.01%), eugenol (0.01%) + thymol (0.01%), thymol (0.01%) + carvacrol (0.01%), isoeugenol (0.01%) + carvacrol (0.01%), isoeugenol (0.01%) + eugenol (0.01%), isoeugenol (0.01%) + thymol (0.01%).

Table 1
Use of 4 organic substances utilized for determining level of survival of nematodes in the laboratory experiment

Name, CAS number, formula	Plants whose essential oil contains this substance and their use	Information on the toxicity of the substance delivered orally to rats and mice LD_{50}
Thymol (CAS 89-83-8) 	Thymol is found in some essential oils, such as thyme ,extracted from the leaves and seeds of *Thymus vulgaris* (Lamiaceae) in amounts of 20–50%. It is also found in *Acanthospermum australe* (Asteraceae), *Humulus lupulus* (Cannabaceae) and other plants. It is used as a stabilizer in pharmaceuticals. It has antiseptic, antibacterial and antifungal effects.	Rat LD_{50}: oral 980 mg/kg. Mouse LD_{50}: oral 640 mg/kg. Guinea pig LD_{50}: oral 880 mg/kg.
Carvacrol (CAS 499-75-2) 	Carvacrol is present in the essential oils of oregano, thyme, savory, *Monarda* and wild bergamot. Essential oils of thyme and *Monarda* contain from 5% to 75% carvacrol, and savory oil – up to 45%. In addition, it is found in *Callistemon citrinus* (Myrtaceae), *Perilla frutescens* (Lamiaceae) and other plants. It has bactericidal and insecticidal properties.	Rat LD_{50}: oral 810 mg/kg. Mouse LD_{50}: intravenous 80 mg/kg.
Eugenol (CAS 97-53-0) 	Eugenol is the main component of the following essential oils: clove (up to 85%), eugenol basil (70–80%), *Coluria* (70–80%), as well as citronella, ylang-ylang, calamus, *Sassafras* and some others.	Rat LD_{50}: oral 1930 mg/kg. Mouse LD_{50}: oral 3 mg/kg; Intraperitoneal 500 mg/kg. Guinea pig LD_{50}: oral 2130 mg/kg.
Isoeugenol (CAS 97-54-1) 	Isoeugenol is found in clove essential oil, as well as in the oil of eugenol basil and *Coluria*. Clove oil contains mainly cis-isoeugenol, while the oil of eugenol basil and *Coluria* contains trans-isoeugenol.	Rat LD_{50}: oral 1560 mg/kg. Mouse LD_{50}: intraperitoneal 328 mg/kg. Guinea pig LD_{50}: oral 1410 mg/kg.

Larval survival test. A suspension of larvae (0.1 mL) was placed in 1.5 mL test tubes. 1 mL of the studied mixtures was then added in fivefold repetition. The test tubes were placed in a thermostat at a temperature of 22 °C with an exposure of 24 hours. After this, 1 mL of the supernatant was collected with a pipette, and the sediment (0.1 mL) was placed on a glass slide and the numbers of live (mobile larvae) and dead (immobile larvae with destroyed intestinal cells) individuals was counted.

Egg survival test. Nematode eggs were detected by washing with a normal saline followed by centrifugation for 2 minutes at 1500 rpm. A Micromed CM-3M centrifuge (Micromed, Kharkov, Ukraine, 2022) was also used for this purpose.

The sediment with eggs (volume 0.1 mL) was placed in test tubes (1.5 mL) and filled with solutions of the studied substances (1% and 0.1% for *T. vulpis*), as well as their mixtures (0.01% for *T. canis* and *T. vulpis*) in fivefold repetition. The exposure was 24 hours. After this time, the nematode eggs were washed with a standard saline and filled with water at room temperature (18–22 °C) followed by cultivation of eggs (with the formation of larvae) in a thermostat. The temperature in the thermostat was 28 °C. The duration of egg cultivation was 21 days for *T. canis* eggs and 60 days for *T. vulpis* eggs. Eggs with formed larvae were counted as alive, without larvae – as dead.

Statistical analysis. The statistical analysis of the results was performed through a set of Statistica 12 (StatSoft Inc., USA). In the text, the data are presented as average value (x) ± standard deviation (SD). The small square in the figures denotes the median, the lower and upper boundaries of the rectangle correspond to the first and third quartiles, the lower and upper parts of the vertical line – to the minimum and maximum values, circles – outliers; different letters above the samples correspond to reliable differences ($P < 0.05$) between the samples according to the results of the Tukey test.

Results

The mortality of *S. papillosus* larvae (Fig. 2) under the influence of a mixture of 0.01% of the studied substances exceeded 90% for isoeugenol and thymol (97.5 ± 1.7%), eugenol and thymol (96.6 ± 2.6%), thymol and carvacrol (92.0 ± 5.8%). It did not differ significantly, but the average mortality nevertheless became lower than 90%, under the influence of eugenol and isoeugenol (89.1 ± 10.3%), eugenol and carvacrol (88.2 ± 8.5%). The minimum mortality of *S. papillosus* larvae was recorded for a mixture of 0.01% isoeugenol and carvacrol (74.5 ± 5.0%). Mortality in the control variant of the experiment (without the effect of organic substances) in *S. papillosus* larvae differed insignificantly (11.8 ± 8.7%) from the mortality of *H. contortus* larvae (6.3 ± 6.9%) (Fig. 2 and 3).

Haemonchus contortus larvae were significantly more resistant to the effect of 0.01% mixtures of the studied substances (Fig. 3). The mixtures of isoeugenol and thymol (7.0 ± 6.8%), isoeugenol and carvacrol (6.5 ± 5.8%) did not exceed the value for the control variant of the experiment (6.3 ± 6.9%). Mortality of *H. contortus* larvae increased for the mixture of eugenol and carvacrol (36.2 ± 6.8%), thymol and carvacrol (59.4 ± 8.8%). The highest mortality of *H. contortus* larvae was observed for eugenol and isoeugenol (80.0 ± 10.6%), eugenol and thymol (86.8 ± 9.6%).

When studying the effects of thymol, carvacrol, eugenol, isoeugenol, the mortality of nematode eggs (*T. vulpis*) in the control variant of the experiment (without the effect of experimental organic substances) was 21.4 ± 6.1% (Fig. 4). The maximum mortality rates under the influence of 0.1% of the studied substances were recorded for carvacrol (80.2 ± 11.4%), significantly lower – for thymol (55.3 ± 7.5%), and remained at the level of the control group (without the effect of any substances) for eugenol (31.6 ± 8.8%) and isoeugenol (25.6 ± 8.0%). The average mortality of *T. vulpis* eggs exceeded 90% when exposed to 1.0% thymol (98.6 ± 1.3%), eugenol (93.0 ± 6.6%), and isoeugenol (92.6 ± 7.4%). The minimum mortality (86.8 ± 7.2%) for *T. vulpis* eggs exposed to the four studied substances was shown for carvacrol (Fig. 4).

At the last stage of the experiment, when studying the degree of influence of the mixtures of thymol, carvacrol, eugenol, isoeugenol on the viability of nematode eggs, eggs of two species of carnivorous nematodes (*T. vulpis* and *T. canis*) were used. They have a high survival level in the environment due to their thick shell. The maximum mortality of *T. vulpis* eggs was observed when exposed to a mixture of isoeugenol and carvacrol. However, this rate did not exceed 31% (30.2 ± 11.3%). The eggs of this nematode species were even more resistant to the effects of other mixtures: isoeugenol and thymol (19.4 ± 6.3%), eugenol and thymol (19.5 ± 8.5%). Less than 15% mortality in the eggs of this nematode species was recorded when exposed to mixtures of eugenol and carvacrol, thymol and carvacrol, and isoeugenol and eugenol (Fig. 5). The eggs of *T. canis* turned out to be the most resistant to the effects of the mixtures (Fig. 6). The mortality of the eggs of this nematode species did not exceed 16%. The maximum mortality rate was recorded when exposed to a mixture of eugenol and carvacrol (15.2 ± 5.9%), as well as isoeugenol and thymol (14.8 ± 7.7%).

Discussion

Parasitic diseases of animals are one of the biggest health problems in extensive livestock farming, where chemically synthesized antiparasitic agents are routinely used, which enter the environment along with organic waste and have toxic effects. Achieving sustainable pasture ecosystems requires a comprehensive strategy that includes farm monitoring with assessment of ecological, parasitological and other indicators, as well as alternative medicine (Pisseri et al., 2013; Rehman & Abidi, 2022). Our results indicate a significant anthelmintic effect of such natural substances as thymol, carvacrol, eugenol, isoeugenol. The studied components of plant essential oils are food additives that can be used in animal husbandry to prevent infectious diseases (Souza et al., 2022; Thiele-Bruhn et al., 2024). All these components have pronounced antibacterial properties against various pathogenic microorganisms (Alagawany et al., 2020; Posgay et al., 2022). The antibacterial effect of isoeugenol against *Pseudomonas aeruginosa* is also indicated by the studies of Galvão et al. (2022).

Carvacrol and its compounds have a strong antimicrobial effect on many strains of bacteria and fungi that can be dangerous to humans (Mączka et al., 2023). According to Šegvić Klarić et al. (2007), thymol also has antifungal properties. These data are also confirmed by other researchers (Bennis et al., 2004; Polanska et al., 2021).

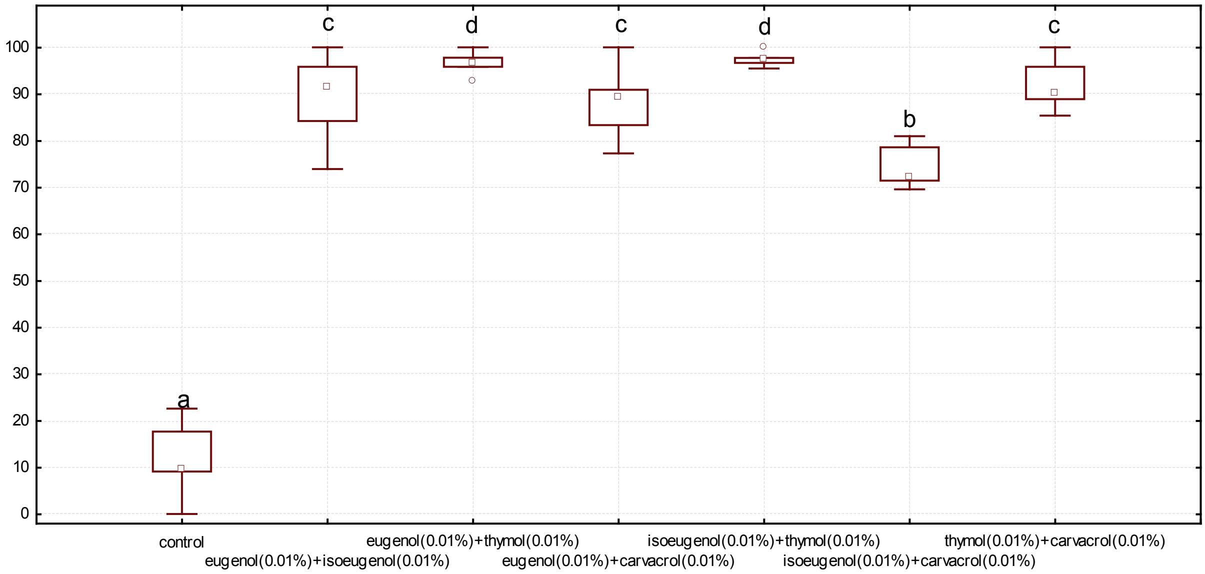


Fig. 2. Mortality (%) of first and second age larvae of *Strongyloides papillosus* exposed to a mixture of two organic components at a concentration of 0.01% for 24 hours (n = 5): small square – median, lower and upper boundaries of the rectangle correspond to the first and third quartiles, lower and upper parts of the vertical line – minimum and maximum values, circle – outliers; different letters above the samples correspond to significant differences (P < 0.05) between the samples according to the results of the Tukey test

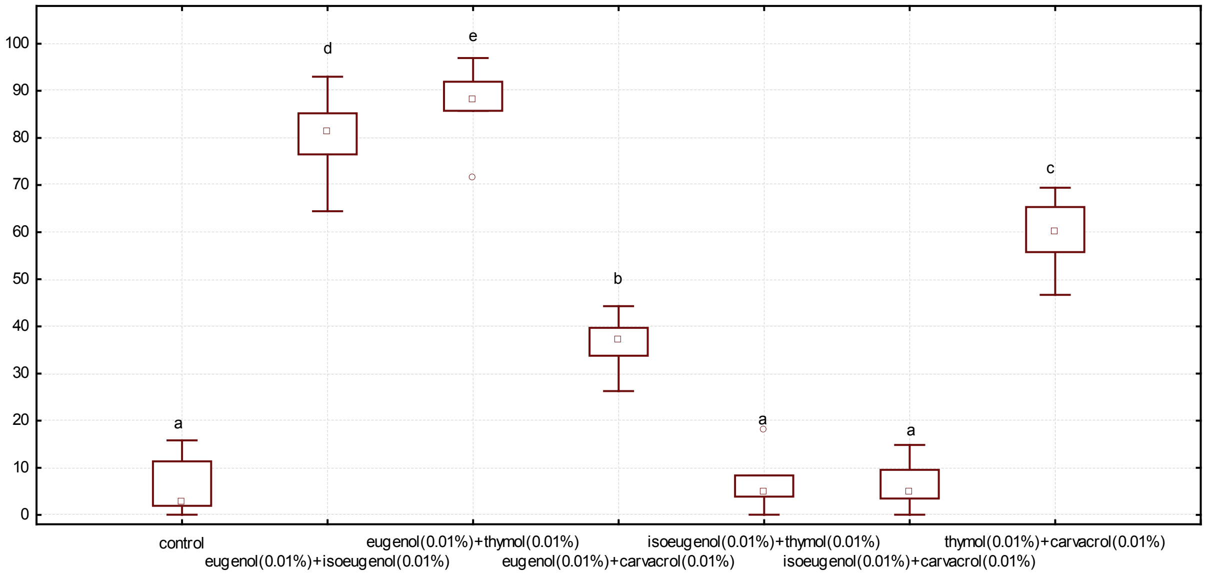


Fig. 3. Mortality (%) of third age larvae of *Haemonchus contortus* following exposure to a mixture of two organic components at a concentration of 0.01% for 24 h (n = 5): for explanation see Fig. 2

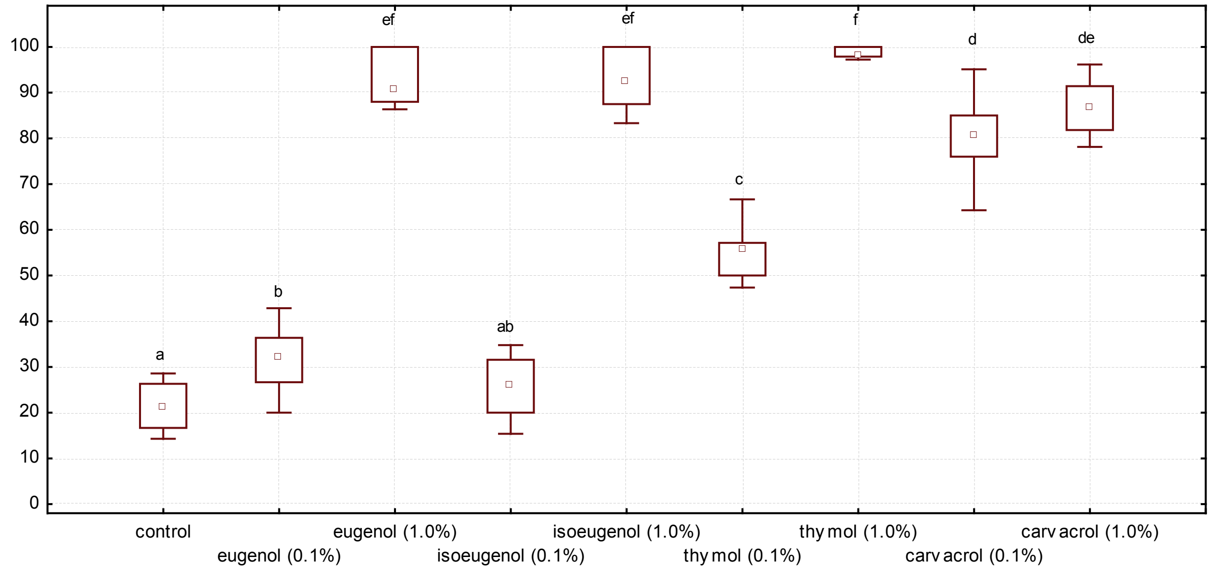


Fig. 4. Mortality (%) of *Trichiuris vulpis* eggs exposed to 0.1% and 1.0% organic substances for 24 hours (n = 5): for explanation see Fig. 2

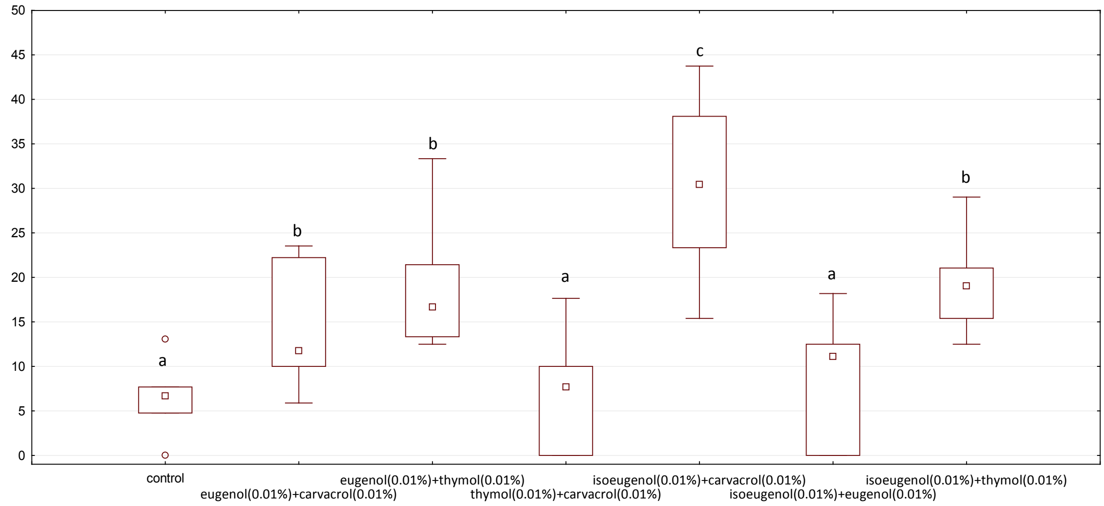


Fig. 5. Mortality (%) of *Trichuris vulpis* eggs following exposure to a mixture of two organic components at a concentration of 0.01% for 24 hours (n = 5): for explanations see Fig. 2

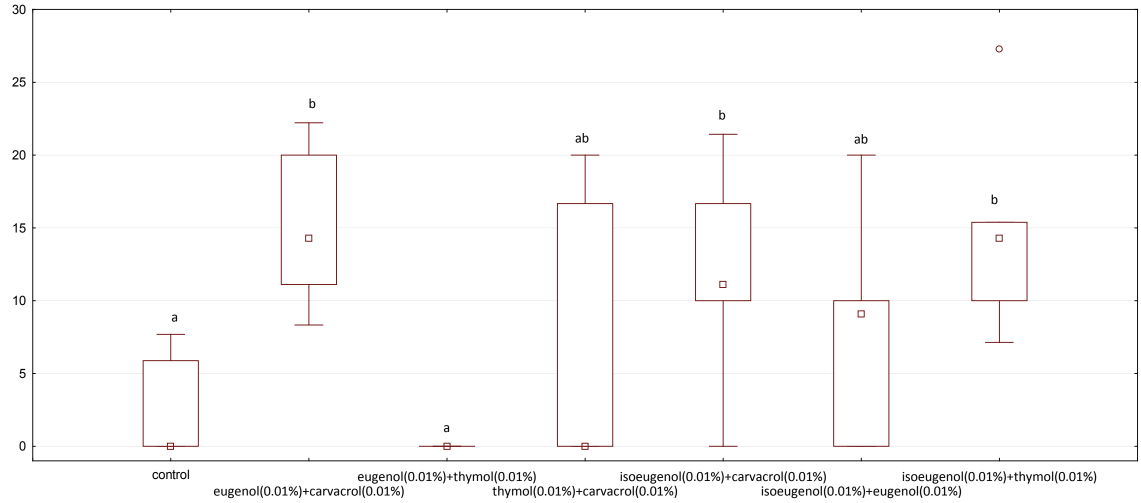


Fig. 6. Mortality (%) of *Toxocara canis* eggs following exposure to a mixture of two organic components at a concentration of 0.01% for 24 hours (n = 5): for explanations see Fig. 2

Thymol and carvacrol are also used to combat parasitic arthropods such as *Varroa destructor* Anderson & Trueman, 2000 (Mesostigmata, Varroidae) (Brasesco et al., 2017). The insecticidal properties of carvacrol are also described by Giatropoulos et al. (2022). The results of their research indicate that carvacrol is a fairly effective larvicide against *Aedes albopictus* (Skuse, 1894) (Diptera, Culicidae). Eugenol and isoeugenol also have insecticidal properties (Huang et al., 2002).

According to the results of our previous studies, even minor concentrations (0.1–1.0%) of most of these substances can lead to the death of more than 90% of nematode larvae, dangerous parasites of the gastrointestinal tract of ruminants. At the same time, their mixtures are even more active against nematode larvae. 0.01% mixture of these substances also has pronounced anthelmintic properties. When nematode larvae were exposed separately to 0.01% solutions of each substance, the death of only a small proportion of specimens (less than 50%) was previously observed within 24 hours. Exposure to mixtures of thymol, carvacrol, eugenol, isoeugenol at the same concentration led to the death of more than 70% of *S. papillosus*. *H. contortus* larvae are more resistant to various environmental factors, including organic and inorganic substances, as described previously (Boyko & Brygadyrenko, 2019b). But mixtures such as eugenol and isoeugenol, as well as eugenol and thymol, led to the death of more than 80% of the larvae of this species of nematode.

The most resistant among the eggs of nematodes in the environment are those that have a very dense and thick shell (Thienpont et al., 2003). These are the eggs of nematodes of the Ascarididae family, including parasites of carnivores of the genus *Toxocara* Stiles & Hassall, 1905 (Zajac, 2011; Boyko et al., 2024). Representatives of the Trichuridae family, including representatives of the genus Trichuris Roederer, 1761 (Yevstafieva et al., 2019), are also resistant to various environmental factors.

Conclusions

The sustainability of pasture ecosystems is achieved by a comprehensive strategy that includes alternative methods of controlling helminthic diseases. The use of mixtures of plant substances such as thymol, carvacrol, eugenol and isoeugenol has shown a pronounced negative effect on the development and viability of eggs and larvae of animal helminths. The studied substances and their mixtures had a lesser effect on eggs than on nematode larvae. Although 1% concentration of these substances had a significant effect on the formation of larvae in eggs, their mixtures in less concentrated solutions did not give a significant result. Eugenol, isoeugenol, thymol, and carvacrol have pronounced anthelmintic properties against nematode larvae of the species *Strongyloides papillosus* and *Haemonchus contortus*, as well as against *Trichiuris vulpis* and *Toxocara canis* eggs. At the same time, an effective synergistic effect on the survival of larvae of the studied nematode species was recorded for mixtures of eugenol and carvacrol, eugenol and thymol, thymol and carvacrol, isoeugenol and carvacrol, isoeugenol and eugenol, isoeugenol and thymol at an insignificant concentration of 0.01%. The most pronounced anthelmintic properties against nematode larvae were recorded for isoeugenol and thymol ($97.5 \pm 1.7\%$), eugenol and thymol ($96.6 \pm 2.6\%$), and thymol and carvacrol ($92.0 \pm 5.8\%$). No effect of 0.01% solutions of the mixtures on the viability of *T. vulpis* and *T. canis* eggs was observed. More than 68% of eggs after 24-hour treatment with the mixtures formed viable larvae.

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The authors declare no conflict of interest.

References

Agrawal, V., Tiwari, S. P., Choudhary, N. S., & Shrivastava, N. (2024). Technological advances for sustainable helminth control in animal health amidst changing climate. Indian Journal of Animal Health, 63(2S), 31–42.

Alagawany, M., Farag, M. R., Abdelnour, S. A., & Elnesr, S. S. (2020). A review on the beneficial effect of thymol on health and production of fish. Reviews in Aquaculture, 13(1), 632–641.

Alves Resende, J., Soares Toneto, D., Cruz Albuquerque, M. C., Areas Bastos, K., Fontes Pinheiro, P., & Drummond Costa Ignacchiti, M. (2022). Antibacterial and anti-biofilm potential of *Plectranthus amboinicus* (Lour.) Spreng essential oil and carvacrol against *Staphylococcus aureus* and *Escherichia coli*. Revista de Ciências Médicas e Biológicas, 21(1), 11–17.

Barbosa, L. N., Alves, F. C. B., Andrade, B. F. M. T., Albano, M., Rall, V. L. M., Fernandes, A. A. H., Buzalaf, M. A. R., Leite, A. de L., de Pontes, L. G., dos Santos, L. D., & Fernandes Jr., A. (2020). Proteomic analysis and antibacterial resistance mechanisms of *Salmonella enteritidis* submitted to the inhibitory effect of *Origanum vulgare* essential oil, thymol and carvacrol. Journal of Proteomics, 214, 103625.

Bennis, S., Chami, F., Chami, N., Bouchikhi, T., & Remmal, A. (2004). Surface alteration of *Saccharomyces cerevisiae* induced by thymol and eugenol. Letters in Applied Microbiology, 38(6), 454–458.

Biswal, R. A., & Pazhamalai, V. (2021). Chapter 13 – Thymol. In: Mushtaq, M., & Anwar, F. (Eds.). A centum of valuable plant bioactives. Academic Press. Pp. 275–290.

Boyko, A. A., & Brygadyrenko, V. V. (2017). Changes in the viability of the eggs of *Ascaris suum* under the influence of flavourings and source materials approved for use in and on foods. Biosystems Diversity, 25, 162–166.

Boyko, O. & Brygadyrenko, V. (2023). Survival of nematode larvae after treatment with eugenol, isoeugenol, thymol, and carvacrol. Frontiers in Bioscience-Elite, 15, 25.

Boyko, O. O., & Brygadyrenko, V. V. (2018). The impact of certain flavourings and preservatives on the survivability of larvae of nematodes of Ruminantia. Regulatory Mechanisms in Biosystems, 9, 118–123.

Boyko, O. O., & Brygadyrenko, V. V. (2019a). Nematocidial activity of aqueous solutions of plants of the families Cupressaceae, Rosaceae, Asteraceae, Fabaceae, Cannabaceae and Apiaceae. Biosystems Diversity, 27, 227–232.

Boyko, O. O., & Brygadyrenko, V. V. (2019b). The impact of acids approved for use in foods on the vitality of *Haemonchus contortus* and *Strongyloides papillosus* (Nematoda) larvae. Helminthologia, 56, 202–210.

Boyko, O. O., & Brygadyrenko, V. V. (2020). The impact of certain flavourings and preservatives on the survivability of eggs of *Ascaris suum* and *Trichuris suis*. Regulatory Mechanisms in Biosystems, 11, 344–348.

Boyko, O. O., Kabar, A. M., & Brygadyrenko, V. V. (2020). Nematicidal activity of aqueous tinctures of medicinal plants against larvae of the nematodes *Strongyloides papillosus* and *Haemonchus contortus*. Biosystems Diversity, 28, 119–123.

Boyko, O., & Brygadyrenko, V. (2021). Nematicidal activity of essential oils of medicinal plants. Folia Oecologica, 48, 42–48.

Boyko, O., & Brygadyrenko, V. (2022). Nematicidal activity of organic food additives. Diversity, 14, 615.

Boyko, O., Brygadyrenko, V., Chernysh, Y., Chubur, V., & Roubík, H. (2024). Possibilities of decontaminating organic waste from swine-farming complexes using anaerobic digestion. Biomass Conversion and Biorefnery, 15(7), 11175–11183.

Boyko, O., Shendryk, L., Shaban, O., & Brygadyrenko, V. (2021). Influence of essential oils on sporulation of *Eimeria magna* oocysts. Annals of Parasitology, 67, 11–17.

Brasesco, C., Gende, L., Negri, P., Szawarski, N., Iglesias, A., Eguaras, M., Ruffinengo, S., & Maggi, M. (2017). Assessing in vitro acaricidal effect and joint action of a binary mixture between essential oil compounds (thymol, phellandrene, eucalyptol, cinnamaldehyde, myrcene, carvacrol) over ectoparasitic mite *Varroa destructor* (Acari: Varroidae). Journal of Apicultural Science, 61(2), 203–215.

Donald, A. D. (1994). Parasites, animal production and sustainable development. Veterinary Parasitology, 54(1–3), 27–47.

Galvão, J. L. F. M., Rosa, L. L. S., Diniz Neto, H., Silva, D. de F., Nóbrega, J. R., Cordeiro, L. V., Figueiredo, P. T. R. de, Andrade Júnior, F. P. de, Oliveira Filho, A. A. de, & Lima, E. de O. (2022). Antibacterial effect of isoeugenol against *Pseudomonas aeruginosa*. Brazilian Journal of Pharmaceutical Sciences, 58, e20075.

Giatropoulos, A., Karamaouna, F., Ampatzi, A., Papachristos, D., & Michaelakis, A. (2022). Sublethal effects of oregano essential oil and its major compound carvacrol on biological parameters of *Aedes albopictus* (Diptera: Culicidae). Experimental Parasitology, 242, 108392.

Huang, Y., Ho, S.-H., Lee, H.-C., & Yap, Y.-L. (2002). Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Journal of Stored Products Research, 38(5), 403–412.

Kowalczyk, A., Przychodna, M., Sopata, S., Bodalska, A., & Fecka, I. (2020). Thymol and thyme essential oil – new insights into selected therapeutic applications. Molecules, 25(18), 4125.

Mączka, W., Twardawska, M., Grabarczyk, M., & Wińska, K. (2023). Carvacrol – A natural phenolic compound with antimicrobial properties. Antibiotics, 12(5), 824.

Marchese, A., Orhan, I. E., Daglia, M., Barbieri, R., Di Lorenzo, A., Nabavi, S. F., Gortzi, O., Izadi, M., & Nabavi, S. M. (2016). Antibacterial and antifungal activities of thymol: A brief review of the literature. Food Chemistry, 210, 402–414.

Mondal, A., Bose, S., Mazumder, K., & Khanra, R. (2020). Carvacrol (*Origanum vulgare*): Therapeutic properties and molecular mechanisms. In: Pal, D., & Nayak, A. K. (Eds.). Bioactive natural products for pharmaceutical applications. Springer, Cham. Pp. 437–462.

Morgan, E., Charlier, J., Hendrickx, G., Biggeri, A., Catalan, D., Von Samson-Himmelstjerna, G., Demeler, J., Müller, E., Van Dijk, J., Kenyon, F., Skuce, P., Höglund, J., O'Kiely, P., Van Ranst, B., De Waal, T., Rinaldi, L., Cringoli, G., Hertzberg, H., Torgerson, P., … Vercruysse, J. (2013). Global change and helminth infections in grazing ruminants in Europe: Impacts, trends and sustainable solutions. Agriculture, 3(3), 484–502.

Pisseri, F., de Benedictis, C., Roberti di Sarsina, P., & Azzarello, B. (2013). Sustainable animal production, systemic prevention strategies in parasitic diseases of ruminants. Alternative and Integrative Medicine, 2(2), 1000106.

Polanska, V. P., Fedorchenko, V. I., Zachepilo, S. V., & Bobrova, N. A. (2021). Effect of eugenol emulsion in polysorbate-80 on clinical strains of *Candida albicans*. Eastern Ukrainian Medical Journal, 9(3), 238–246.

Posgay, M., Greff, B., Kapcsándi, V., & Lakatos, E. (2022). Effect of *Thymus vulgaris* L. essential oil and thymol on the microbiological properties of meat and meat products: A review. Heliyon, 8(10), e10812.

Rathod, N. B., Kulawik, P., Ozogul, F., Regenstein, J. M., & Ozogul, Y. (2021). Biological activity of plant-based carvacrol and thymol and their impact on human health and food quality. Trends in Food Science and Technology, 116, 733–748.

Rehman, A., & Abidi, S. M. A. (2022). Chapter 29 – Livestock health: Current status of helminth infections and their control for sustainable development. In: Sobti, R. C. (Ed.). Advances in animal experimentation and modeling. Elsevier. Pp. 365–378.

Resende Alves, J., Toneto Soares, D., Albuquerque Cruz, M. C., Bastos Areas, K., Pinheiro Fontes, P., & Ignacchiti Drummond Costa, M. (2022). Antibacterial and anti-biofilm potential of *Plectranthus amboinicus* (Lour.) Spreng essential oil and carvacrol against *Staphylococcus aureus* and *Escherichia coli*. Revista de Ciências Médicas e Biológicas, 21(1), 11–17.

Salehi, B., Mishra, A. P., Shukla, I., Sharifi-Rad, M., Contreras, M. D. M., Segura-Carretero, A., Fathi, H., Nasrabadi, N. N., Kobarfard, F., & Sharifi-Rad, J. (2018). Thymol, thyme, and other plant sources: Health and potential uses. Phytotherapy Research, 32(9), 1688–1706.

Šegvić Klarić, M., Kosalec, I., Mastelić, J., Piecková, E., & Pepeljnak, S. (2007). Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. Letters in Applied Microbiology, 44(1), 36–42.

Souza, V. V. M. A., Almeida, J. M., Barbosa, L. N., & Silva, N. C. C. (2022). Citral, carvacrol, eugenol and thymol: Antimicrobial activity and its application in food. Journal of Essential Oil Research, 34(3), 181–194.

Thanh, N. P. T., Kim, X. V., Kim, M.-T. N., & Tran, T. T. (2024). Extracting and evaluating the antibacterial activity of carvacrol from *Plectranthus amboinicus* essential oil against *Staphylococcus aureus* and MRSA. IOP Conference Series: Earth and Environmental Science, 1340(1), 012030.

Thiele-Bruhn, S., Shikuku, V., Dittrich, F., Torjir, D. N., Saini, M., & Getenga, Z. (2024). Soil sorption and effects on soil microorganisms of thymol and carvacrol monoterpenes from essential oils of aromatic plants. Frontiers in Environmental Science, 12, 1379018.

Thienpont, D., Rochette, F., & Vanparijs, O. F. J. (2003). Diagnosing helminthiasis by coprological examination. Third edition. Janssen Animal Health, Beerse.

Topal, F. (2019). Anticholinergic and antidiabetic effects of isoeugenol from clove (*Eugenia caryophylata*) oil. International Journal of Food Properties, 22(1), 583–592.

Van Wyk, J. A., & Mayhew, E. (2013). Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. Onderstepoort Journal of Veterinary Research, 80(1), 539.

Van Wyk, J., Cabaret, J., & Michael, L. (2004). Morphological identification of nematode larvae of small ruminants and cattle simplified. Veterinary Parasitology, 119(4), 277–306.

Wang, Q., Mukesh Kumar Awasthi, M. K., Ren, X., Zhao, J., Wang, M., Chen, H., & Zhang, Z. (2018). Recent advances in composting of organic and hazardous waste: A road map to safer environment. In: Varjani, S., Parameswaran, B., Kumar, S., & Khare, S. (Eds.). Biosynthetic technology and environmental challenges. Energy, environment, and sustainability. Springer, Singapore. Pp. 307–329.

Xie, Y., Yang, Z., Cao, D., Rong, F., Ding, H., & Zhang, D. (2015). Antitermitic and antifungal activities of eugenol and its congeners from the flower buds of *Syzgium aromaticum* (clove). Industrial Crops and Products, 77, 780–786.

Yevstafieva, V. A., Kravchenko, S. O., Gutyj, B. V., Melnychuk, V. V., Kovalenko, P. N., & Volovyk, L. B.(2019). Morphobiological analysis of *Trichuris vulpis* (Nematoda, Trichuridae), obtained from domestic dogs. Regulatory Mechanisms in Biosystems, 10(2), 165–171.

Zajac, A. M., & Conboy, G. A. (Eds.). (2011). Veterinary clinical parasitology. 8th ed. John Wiley and Sons, London.