The impact of certain flavourings and preservatives on the survivability of larvae of nematodes of Ruminantia

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Introduction

Throughout the world a daily struggle takes place against parasitic diseases of agricultural animals. These infestations cause significant economic damage to farming enterprises and large stock-raising facilities, which consequently fall short of their potential in meat and dairy production. The most common parasitic diseases are helminthiases (Vercruysse et al., 2001; Biffa et al., 2001; Bhutto et al., 2002). Some of the above mentioned parasitic nematodes of Ruminantia animals. Among these substances, the lowest efficiency against the nematode larva was found in isoamyl alcohol, isoamyl acetate and trilon B. A stronger effect was caused by methylparaben (for L3 of *S. papillosus* LD₅₀ = 0.67 ± 0.04%, L₁ and L₂ *S. papillosus* – LD₅₀ = 0.0038 ± 0.0008%, L₁ *H. contortus* – LD₅₀ = 0.89 ± 0.15%). Minimum efficient dosage of the solutions was 10 g/l. Significant anthelmintic properties were manifested by raspberry ketone (for L₁ *S. papillosus* LD₅₀ = 1.00 ± 0.72%, L₁ and L₂ *S. papillosus* – LD₅₀ = 0.07 ± 0.06%, L₁ *H. contortus* – LD₅₀ = 0.39 ± 0.26%). The results show that there is considerable potential for further studies on the antiparasitic properties of these substances against nematodes in the conditions of farming enterprises and agricultural complexes.

Keywords: Strongyloides papillosus; Haemonchus contortus; antiparasitic activity; flavouring agents; isoamyl alcohol; isoamyl acetate; raspberry ketone; Trilon B; methylparaben

Materials and methods

In the summer of 2017, we collected faeces of Ruminantia on the territory of Dnipropetrovsk oblast of Ukraine to the amount of 100 g from every individual (n = 56). The material was transported in plastic containers at a temperature of 22–24 °C to the parasitological laboratory of Dnipro State Agrarian-Economic University. The samples with helminths for use in the experiment were taken on the territory of Dnipropetrovsk oblast of Ukraine to the amount of 100 g from every individual (n = 56).
identified using the McMaster method. For the study, we selected third age larvae (L₃) of *Haemonchus contortus* (Rudolphi, 1803) from the Strongylida order and larvae of first, second and third age (L₁, L₂, L₃) of *Strongyloides papillosus* (Wedl, 1856) from the Rhabditida order (Van Wyk & Mayhew, 2013) (Fig. 1). For the experiment, the larvae were cultivated during 8 days at a temperature of 22–24 °C. The larvae material was collected using the Baermann test (Zajac & Conboy, 2011).

**Table 1**
Usage and properties of the flavourings and preservatives* used for determining the level of survivability of *Strongyloides papillosus* (Wedl, 1856) and *Haemonchus contortus* (Rudolphi, 1803) larvae

<table>
<thead>
<tr>
<th>Substance name</th>
<th>Chemical formula</th>
<th>Structural formula</th>
<th>Properties</th>
<th>Content</th>
<th>Usage in food industry</th>
<th>Usage in medicine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoamyl alcohol</td>
<td>C₅H₁₂O</td>
<td><img src="image" alt="Isoamyl alcohol" /></td>
<td>optically inactive colourless substance with unpleasant odour</td>
<td>fused oils</td>
<td>used for preparing extractions with pleasant fruit odour</td>
<td>no data on usage</td>
</tr>
<tr>
<td>Isoamyl acetate</td>
<td>C₇H₁₄O₂</td>
<td><img src="image" alt="Isoamyl acetate" /></td>
<td>colourless substance with sharp pear odour</td>
<td>in some fruits</td>
<td>pear extraction for producing fruit water, caramel, etc.</td>
<td>no data on usage</td>
</tr>
<tr>
<td>Raspberry ketone</td>
<td>C₁₀H₁₂O₂</td>
<td><img src="image" alt="Raspberry ketone" /></td>
<td>colourless substance with citrus odour</td>
<td>in red raspberries</td>
<td>as a food additive with fruit flavour</td>
<td>used in cosmetology</td>
</tr>
<tr>
<td>Triton B</td>
<td>C₁₀H₂₂N₂S₄O₈</td>
<td><img src="image" alt="Triton B" /></td>
<td>white crystalline powder or crystals of white colour</td>
<td>–</td>
<td>in food preservation, as antioxidant</td>
<td>in the production of medical preparations and in cases of heavy metal intoxication, in dentistry, as a preservative in eye preparations</td>
</tr>
<tr>
<td>Methylparaben, E₂₁₈</td>
<td>C₆H₁₂O₃</td>
<td><img src="image" alt="Methylparaben" /></td>
<td>white crystalline substance with distinctive odour</td>
<td>in the roots of <em>Oxalis tuberosa</em></td>
<td>as a preservative</td>
<td>as an antiseptic</td>
</tr>
</tbody>
</table>

*Note:* * – properties of the substances are given according to Lide, 1980; Fahrbusch et al., 2002; Soni et al., 2002; Catalog of Organics and Fine Chemicals, 2004; Nomenclature of Organic Chemistry, 2014.
Sediment with larvae was obtained by centrifugation (4 minutes at 1500 circles per minute), which was put into 1.5 ml plastic test tubes in equal portions. Then 1 ml of 1.0% water solution of each tested substance was added to the larvae cultures (0.1 ml, 20–40 ind.). The exposure lasted 24 hours. The temperature regime in the thermostat was within 22–24 °C. The nematode larvae were affected by food additives from the group of flavourings, and also preservatives (the experiment used three concentrations of the tested substances: 1%, 0.01%, 0.0001%). Every variant of the experiment was repeated eight times. The laboratory studies were conducted using chemically pure isoamyl alcohol, isoamyl acetate, raspberry ketone, Trilon B, and methylparaben (Table 1).

The statistical analysis of the results was performed through a set of Statistica 8.0 (StatSoft Inc., USA), the figures is show the median, 25% and 75% quartiles, minimum and maximum values. LD50 (%) was calculated as average (x) ± standard deviation (SD).

**Results**

The results indicated a complete absence of anthelmintic properties in isoamyl alcohol (Fig. 2a) and isoamyl acetate. With exposure to isoamyl alcohol, we observed around 60% vital larvae of L3 *S. papillosus* in 1% solution. Less resistant to the impact of isoamyl alcohol were L1 and L2 *S. papillosus*. The percentage of the surviving larvae of these two stages after 24 hours of exposure to 1% solution was 55%. With further solutions of isoamyl alcohol, 80% of *S. papillosus* larvae survived. Larvae of L3 *H. contortus* were found to be the most resistant to different concentrations of this substance. At 0.0001–1% solution of this alcohol, 100% of them survived.

The next flavouring, isoamyl acetate, manifested the weakest influence on the mortality of nematode larvae of Ruminantia (Fig. 2b). In 1% solution, almost all larvae of *S. papillosus* and *H. contortus* survived. Similarly, at 0.01% and 0.0001% concentrations of this substance, most of the larvae of all studied nematode species survived.

1% solution of raspberry ketone caused 100% mortality only to L1 and L2 *S. papillosus*. 30–50% of L3 *S. papillosus* and *H. contortus* survived in this concentration. In 0.01% solution of this substance, over 80% of the Ruminantia nematode larvae survived. 0.0001% concentration of raspberry ketone in 100% of cases did not affect the survivability of these parasites (Fig. 2c).

Fig. 2. The effect of flavourings on the survivability of Ruminantia nematodes: *a* – isoamyl alcohol, *b* – isoamyl acetate, *c* – raspberry ketone; the ordinate axis shows the percentage of nematode larvae which survived over the 24-hour experiment; the abscissa axis shows the concentration of the active substance in the solution (%), K – control, where the concentration of the active substance equals 0%; L3 – invasive larvae of *S. papillosus* or *H. contortus*, L1, L2 – non-invasive larvae of *S. papillosus*; the small square in the center corresponds to the median, the lower and upper borders of the large rectangular correspond to the first and the third quartiles, respectively, vertical line segments, directed up and down from the rectangular, correspond to minimum and maximum values (n = 8)

The second stage of the experiment was determining the anthelmintic properties of some preservatives (trilon B and methylparaben). About 60% of L1 and L2 *S. papillosus* larvae died after 24 hours in 1% solution of trilon B. At the same concentration, all the rest of larvae survived in the percentage of 75–100%. The most resistant to 1% solution of trilon B were the larvae of *H. contortus*.
(100% larvae survived). The next solutions of trilon B had no positive effects – all larvae survived (Fig. 3a). The solution of methylparaben in 1% and 0.01% concentrations caused death of non-invasive S. papillosus (Fig. 3b) larvae in 100% of cases. Invasive larvae of this species, similarly to H. contortus larvae are sensitive to methylparaben only at its maximum, 1% concentration of the active substance. The analysis of the study results indicated complete absence of anthelmintic properties in isoamyl alcohol and trilon B. Minimum LD50 (%) indicators for L3 S. papillosus were registered for isoamyl acetate, raspberry ketone and methylparaben (Table 2).

![Fig. 3. The effect of trilon B (a) and methylparaben (b) on the survivability of Ruminantia nematode larvae: see notes to Fig. 1](image)

<table>
<thead>
<tr>
<th>Substance</th>
<th>S. papillosus</th>
<th>L3 S. papillosus</th>
<th>L1 + L2 S. papillosus</th>
<th>H. contortus, L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>isoamyl alcohol</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>isoamyl acetate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>raspberry ketone</td>
<td>1.00 ± 0.72</td>
<td>0.07 ± 0.06</td>
<td>0.39 ± 0.26</td>
<td>–</td>
</tr>
<tr>
<td>trilon B</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>methylparaben</td>
<td>0.67 ± 0.04</td>
<td>0.0038 ± 0.0008</td>
<td>0.89 ± 0.15</td>
<td>–</td>
</tr>
</tbody>
</table>

Discussion

Therefore, according to the results of our studies and analysis of the data from the literature, the additives used in the food industry can affect parasites, including nematode larvae of Ruminantia, in a certain concentration. Data on using food additives with the purpose of affecting the parasites are quite limited. Their impact on parasitic Acari and insects has been studied by Lee et al. (2008), Knoblach and Fry (2011), Shen et al. (2012), Belkind et al. (2013) et al. Shen et al. (2012) indicate the significant effect of cinnamaldehyde against parasitic Acari Psoroptes. LD50 equals 107 mg/ml, (with 48-hour exposure) for Acari of this genus. Also, this food additive has been studied by Na et al. (2011) as an acaricide preparation against Dermanyssus birds. LD50 for Dermanyssus sp. was 0.54 mg/ml (with 24-hour exposure). According to Lee (2004), p-anisaldehyde food additive is capable of acaricide properties. Other works are devoted to the use of the food additive cinnamaldehyde on larvae of blood-sucking insects. LD50 for larvae of mosquitoes was 40.8 mg/ml. Taylor (2009) used benzyl alcohol against fleas and also indicated that this additive has insecticidal properties. Benzaldehyde has been proved to have impact on insects. It was used against Galleria mellonella (Linnaeus, 1758). The authors of these studies, Ullah et al. (2015), recommended the additive for the compound of insecticidal preparations. Lee et al. (2008) have also used benzaldehyde (LD50 with 48-hour exposure – 0.004–0.200 mg/sm² against Sitophilus oryzae (Linnaeus, 1763) (Coleoptera, Curculionidae). Anthelmint properties of benzyl alcohol additive were proved by Chalquest (2002). Pedersen and Woldum (2011) recommend using it as solvent of preparations against parasites.

Food additives are often used as antimicrobial agents. Their impact on microorganisms has been studied by Chiang et al. (2005), Sato et al. (2006), Somolinos et al. (2008), Si et al. (2009), Belletti et al. (2010) and many other authors. Ribeiro et al. (2016) have studied antimicrobial, antifungi, and also insecticidal impact of 83 compounds from different tissues of Ricinus communis. Some of them are used as additives in the food industry. They include alkaloids, terpenoids, flavonoids, benzoic acid derivatives, coumarins, tocopherols, and fatty acids. The antimicrobial properties of cinnamaldehyde against Escherichia coli and Salmonella entericu were studied by Manu (2016). For obtaining antiseptic and fungidal effect, Е218, a methylparaben preservative, is used (Shapiro et al., 2002; Posey et al., 2005; Kromidas et al., 2006; Rebbeck et al., 2006; Ishiwatari et al., 2007; Meyer et al., 2007; Gopalakrishnan et al., 2012). It is also used in the composition of insecticides (Bell, 1990). According to the results of our studies, this substance also affects other parasitic nematodes of Ruminantia.

The impact of ethylenediaminetetraacetic acid (EDTA or trilon B) on Cryptococcus has been studied by Lai et al. (2016). Currently, fungal diseases are difficult to treat, and such treatment is conducted using expensive preparations. Therefore, these authors’ work was aimed at intensifying the effect of modern preparations by using them with ethylenediaminetetraacetic acid and other synergic agents for decreasing the therapeutic dose, increasing the efficiency and preventing development of Cryptococcus resistance. The results of our experiments indicated that usage of trilon B did not cause any death of parasitic nematodes. Our study also indicates a

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high level of anthelmintic impact of methylparaben and raspberry ketone. These substances affect not only microorganisms and are used as a fungicide, but are also, according to the results of our tests, capable of having an effect on *S. papillosus* and *H. contortus*, parasitic nematodes of Ruminantia.

**Conclusions**

Additives used in the food industry are significant in the struggle against helminthiasis of Ruminantia. Among the flavourings and source materials approved for use in and on foods, raspberry ketone and methylparaben are most efficient against nematode larvae. Minimum efficient dosage of solutions of these substances is 10 g/l or 1% solution.

**References**


Manu, D. K. (2016). Antimicrobial activity of cinnamaldehyde or genzanol alone or combined with high pressure processing to destroy *Escherichia coli O157: H7* and *Salmonella enterica* in juices. Iowa State University, Ames, Iowa.


additives towards *Clostridium perfringens*. Journal of Applied Microbiology, 106(1), 213–220.


