The impact of acids approved for use in foods on the vitality of *Haemonchus contortus* and *Strongyloides papillosus* (Nematoda) larvae

O. O. BOYKO¹, V. V. BRYGADYRENKO¹, ²

¹Dnipro State Agrarian and Economic University, S. Efremova St., 25, Dnipro, 49600, Ukraine, E-mail: boikoalexandra1982@gmail.com; ¹, ²Oles Honchar Dnipro National University, Gagarin Ave. 72, Dnipro, 49010, Ukraine, E-mail: brigad@ua.fm

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**Summary**

The laboratory experiment described in this article evaluated the death rate of larvae of *Haemonchus contortus* (Rudolphi, 1803) nematodes of the Strongylida order and *Strongyloides papillosus* (Wedl, 1856) of the Rhabditida order under the impact of different concentrations of 8 flavouring acids and source materials approved for use in and on foods and in medicine (formic, wine, benzoic, salicylic, stearic, kojic, aminocetic, succinic acids). Minimum LD₅₀ for third stage larvae of (L₃) *S. papillosus* was observed with salicylic and wine acids, for L₃ *H. contortus* larvae – with formic acid. Minimum impact on all studied stages of development of nematodes was caused by stearic, kojic, aminocetic and succinic acids: larvae did not die in the course of one day even at 1 % concentration of these substances. The best parameters of LD₅₀ were observed for benzoic and formic acid. Further experiments on flavouring acids and source materials approved for use in and on foods and in medicines, and also their compounds, will contribute to developing preparations with a stronger impact on nematode larvae – parasites of the digestive tract of vertebrate animals and humans.

**Keywords:** nematodes of ungulates; flavouring acids; death rate of larvae

**Introduction**

Achieving high quality livestock production requires following the rules of maintenance conditions. Important factors are the animals’ diet and measures for preventing infections (Zazharska et al., 2018). One of the most common animal diseases around the world is considered to be helminthiasis. Annually it causes great losses in livestock production and great economic losses. An increasing amount of scientific research is being conducted on preventing and treating agricultural parasites. Farming uses synthetic anthelmintic preparations. Highly popular are broad spectrum anthelmintics: Albendazole, Fenbendazole, Ivermectin preparations (Beloeil et al., 2003; Faye et al., 2003; Fthenakis et al., 2005; Veneziano et al., 2004; Charlier et al., 2007; Cringoli et al., 2008). Also, research on the relative antiparasitic properties of plants is conducted all around the world (Rahmann & Seip, 2006; Burke et al., 2009; Lu et al., 2010). Plant-based medical preparations against helminths show encouraging results. Rahmann and Seip (2006) suggest a list of plants with anthelmintic properties: black cumin, black walnut, boundary tree, common mugwort, common wormwood, crucifers, custard tree, eucalyptus, Eurasian wormwood, fargara, fennel, fen, fumitory garlic, Gambian mahagonya, goosefoot, Indian lilac, kamala tree, neem tree, papaya, pinkroot, pumpkin, pyrethrum, sacred basil, southern wormwood, tansy, tarragon, wild carrot, and wild ginger. A number of authors have studied the impact of sainfoins on the nematodes of animals: feeding sainfoins to goats reduces the number of eggs of *Trichostrongylus* sp. in the animals’ feces (Paolini et al., 2005). Ferreira et al. (2011) described the impact of crude alcoholic extracts of *Artemisia annua*, *A. absinthium*, *Asimi-
Earlier, we tested against nematodes the following flavourings and source materials approved for use in and on foods: p-Anisaldehyde, Benzaldehyde, γ-Undecalactone, Cinnamaldehyde, Ethyl acetate, Benzyl acetate, α-Terpineol, Benzyl alcohol, Citral, L-Linalool, β-Ionone, Citronellol, Acetoïn, D-Limonene (Boyko & Brygadyrenko, 2016). The experiments showed that minimum LD₅₀ for L₂ S. papillosus were achieved using Cinnamaldehyde, α-Terpineol and Benzyl alcohol, for L₁,2 S. papillosus – using Benzyl alcohol, Cinnamaldehyde, L-Linalool and Benzyl acetate, for L₁ H. contortus – using γ-Undecalactone and Cinnamaldehyde. Lowest indicators of LD₅₀ (mg/l) against Strongyloides ransomi Schwartz and Alicata, 1930 were observed using Benzaldehyde (Boyko & Brygadyrenko, 2017b). When invasive eggs of A. suum, were exposed to Cinnamaldehyde, benzoic acid (E210, Codex Alimentarius) and methylparaben (E218, Codex Alimentarius) at 1 % concentration, we determined the lowest parameters of LD₅₀ for benzoic acid (Boyko & Brygadyrenko, 2017a).

Strongyloidiasis and haemonchosis are some of the commonest helminthiases of Ruminantia Ruminantia helminthiases of Strongyloides and haemonchosis are some of the commonest benignac acid (Boyko & Brygadyrenko, 2019). The experiments showed that minimum LD₅₀ for L₂ S. papillosus larvae and first, second and third stage (L₁, L₂, L₃) S. papillosus larvae (Van Wyk et al. 2004; Van Wyk & Mayhew 2013; Boyko et al., 2016). The larvae were obtained using the Baermann test (Zajac & Conboy, 2011). 4 ml of water with larvae was centrifuged for 4 minutes with 1,500 rotations per minute. The centrifuged sediment of liquid with nematode larvae (0.1 ml) was put in plastic test tubes of 1.5 ml capacity. The solutions of acids were added, and the tubes were left in a thermostat for 24 hours at the temperature of +22...+24 °C. The larvae were exposed to the impact of formic, wine, benzoic, salicylic, stearic, kojic, aminoacetic, succinic acids – flavourings and source materials approved for use in and on foods and in medicines. Three concentrations of the substances were used in eightfold replication for every variant of the experiment (Table 1). Benzoic acid is a substance of average toxicity. LD₅₀ (median dose) of benzoic acid for laboratory animals (intravenous administration to rats) equals 1700 mg/kg, for cats – 300 mg/kg (Bedford & Clarke, 1972; Jakimowska, 1961). Formic acid has low toxicity. According to the classification of the European Union (Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances), concentration of formic acid not higher than 10 % has an irritating effect, over 10 % - corrosive. LD₅₀ (median dose) of formic acid for laboratory animals equals 700, 1100, 4000 mg/kg for mice, rats and dogs respectively (oral) (Von Oettingen, 1960, Montgomery, 2000). Wine acid (Tartaric acid) is used in the food industry as a E334 additive (Codex Alimentarius). LD₅₀ is about 5.3 g/kg for rabbits, and 4.4 g/kg for mice (Maga, Tu, 1995). Salicylic acid is toxic for humans only in high doses. LD₅₀ (median dose) of salicylic acid for laboratory animals (mice, intravenous) is 184 mg/kg (Ozawa et al., 1971). Stearic acid is broadly used in cosmetics. LD₅₀ (median dose) of stearic acid for laboratory animals is 21.5 mg/kg (rats, intravenous), 23 mg/kg (mice, intravenous) (Oro & Wretlind, 1961). Kojic acid is broadly used in the food industry, cosmetology, and also medicine. Aminoacetic acid is also used widely. It is used in the food industry (E640), for preparing pharmaceutical preparations, feeders for animals. LD₅₀ (median dose) of aminoacetic acid is 7930, mg/kg (rats, oral) (Wypych, 2016). As a food additive and dietary supplement, succinic acid is generally recognized as safe by the U.S. Food and Drug Administration. This acid is used in the food industry as a E363 additive. Also it is used for obtaining medical preparations. LD₅₀ is 2702mg/kg for laboratory animals (mice, intraperitoneal) (Domingo et al., 1990).

The statistical analysis of the results was performed through a set of Statistica 8.0 (StatSoft Inc., USA). On the figures is shown the median, 25 % and 75 % quartiles, minimum and maximum values. LD₅₀ is expressed as a %: average (x) ± standard deviation (SD).
Ethical Approval and/or Informed Consent

This work does not involve human or experimentation with animals.

Results

The best results were shown by benzoic and formic acids. A total of 100% of L3 *H. contortus* and L1, L2, L3 *S. papillosus* larvae died at 1% concentration of both solutions (Fig. 1 a, b). But about 100% of L3 *S. papillosus* and L3 *H. contortus* L1, L2 survived in the next (0.01%) concentration of benzoic acid. L1, L2 *S. papillosus* were less resistant to 0.01% concentration of benzoic acid – we observed around 70% viable individuals at this concentration.

Under the impact of 0.01% concentration of formic acid, 100% of L3 *S. papillosus* and 100% L3 *H. contortus* remained alive.

The study of the impact of succinic (Fig. 1 d) and aminoacetic acids (Fig. 2 a) also showed a negative anthelmintic effect. About 80% of L3 *S. papillosus* and 100% L3 *H. contortus* remained alive.
Fig. 1. The impact of benzoic (a), formic (b), stearic (c) and succinic (d) acid on vitality of larvae of nematodes of ruminants: the ordinate axis indicates the percentage of living nematode larvae in the course of the 24-hour experiment; the abscissa axis indicates the concentration of the solution’s active substance (%); (K) control, where the concentration of the active substance is 0%; (L3) invasive larvae of *S. papillosus* or *H. contortus*; (L1, L2) non-invasive larvae of *S. papillosus*; the small square in the centre corresponds to the median, the lower and upper edge of the large rectangle corresponds to first and third quartiles, respectively, the vertical segments, directed upward and downward from the rectangles, correspond to minimum and maximum values (n = 8)
Fig. 2. The effect of aminoacetic (a), wine (b), salicylic (c) and kojic (d) acid on vitality of larvae of nematodes of ruminants: explanations see in Fig. 1.
under the impact of aminoacetic acid. 100 % of all invasive L₃ S. papillosus and L₃ H. contortus survived in 1 % solution of succinic acid. L₁, L₂ S. papillosus were less resistant to the acids. Only 15 % and 25 % of the larvae withstood 1 % solution of aminoacetic and succinic acids respectively. The next concentration of aminoacetic acid (0.01 %) also caused positive results only with L₁, L₂ S. papillosus: about 70 % of the larvae died. In 0.01 % solution of succinic acid, the larvae were more resistant: around 60 % survived. Smaller concentrations provided no positive effect against larvae of L₁, L₂ L₃ nematodes of the studied species.

Wine acid showed a less significant nematocidal effect. None of the L₃ H. contortus died even in 1 % concentration of this substance. By contrast, the larvae of S. papillosus of all stages of development died at this concentration. But the next concentration of the wine acid solution (0.01 %) caused mortality of only 40 % of L₃ S. papillosus and 15 % of L₃ S. papillosus. 100 % L₃ of both studied species of nematodes survived at 0.0001 % concentration of this acid (Fig. 2 b).

Exposure to 1 % solution of salicylic acid caused death of only S. papillosus larvae. No more than 20 % of the larvae were able to withstand 0.01 % solution. Exposing S. papillosus larvae to 0.0001 % concentration of salicylic acid did not lead to positive results: more than 70 % of the larvae remained alive. L₃ H. contortus larvae were found to be the most resistant to different concentrations of this acid (Fig. 2 c).

Similar results were obtained after using kojic acid. Over 60 % of L₃ S. papillosus and L₃ H. contortus survived in 1 % solution of this acid. Non-invasive larvae also appeared to be less resistant to the acid: vitality of L₁, L₂ S. papillosus larvae was only 10 % at such concentration. Over 90 % of the larvae of the studied nematode species survived at 0.01 % concentration of kojic acid (Fig. 2 d).

Thus, the best LD₅₀ indicators were observed for benzoic and formic acids. These acids caused death of all studied species of nematode larvae. Stearic, kojic, aminoacetic and succinic acids did not demonstrate a significant impact on the nematode larvae. They increased mortality of only L₁, L₂ S. papillosus (Table 2).

Table 2. LD₅₀ (% ± SD) for S. papillosus and H. contortus larvae in laboratory experiment during 24 hours; (–) the experiment did not achieve death of 50 % of the larvae (over 1 % concentration is needed)

| Substance     | S. papillosus, L₃ | S. papillosus, L₁ + L₂ | H. contortus, L₃ |
|---------------|-------------------|------------------------|------------------|
| benzoic acid  | 0.18 ± 0.14       | 0.07 ± 0.04            | 0.52 ± 0.18      |
| formic acid   | 0.47 ± 0.29       | 0.008 ± 0.007          | 0.41 ± 0.32      |
| wine acid     | 0.008 ± 0.005     | 0.006 ± 0.004          | –                |
| salicylic acid| 0.0010 ± 0.0006   | 0.0009 ± 0.0006        | –                |
| stearic acid  | –                 | 0.09 ± 0.03            | –                |
| kojic acid    | –                 | 0.08 ± 0.03            | –                |
| aminoacetic acid | –              | 0.006 ± 0.004         | –                |
| succinic acid | –                 | 0.08 ± 0.03            | –                |

Discussion

Therefore, flavourings and source materials approved for use in and on foods and acids used in medicine and cosmetology are capable of having a significant effect on vitality of larvae of nematodes of ruminants (S. papillosus, H. contortus). Currently, scientists are closely studying natural factors which are unfavourable for parasites of agricultural animals and plants. The question of using anthelmintic substances of non-synthetic origin against agricultural pests is becoming increasingly relevant. The issues we are interested in, the impacts of flavouring acids and source materials approved for use in and on foods and acids used in medicine and cosmetology, are being reported in the scientific literature. Positive results in using acids against nematodes were also achieved by Browning et al. (2004). They described the nematocidal properties of butyric acid, which is obtained through fermentation of organic substances by anaerobic soil bacteria. A 2-day incubation in sand amended with 0.88 mg/g butyric acid reduced plant parasitic and fungivorous nematodes by 84–100 % as compared to untreated controls. The species compound of nematodes is highly significant. Significant decrease in the number of some species requires using 0.88 mg butyric acid / g of sand, others (Steinernema) require 8.8 mg butyric acid / g of sand in order to effect a significant decline (85 %). Therefore, when formic and benzoic acids are used, much lower concentrations are needed for eliminating nematode larvae (0.01 g/ml) than for butyric acid. Using the gaseous phase of butyric acid against plant nematodes in a 7-day incubation period showed similar results. The vapour from a 0.1 M solution reduced plant-parasitic and fungivorous nematodes by 89–96 % while the vapour from a 1 M solution of butyric acid reduced entomogenous nematodes by 94–99 %.

Browning et al. (2006) have also studied in laboratory conditions the nematocidal properties of butyric acid on fungal and nematode endoparasites of strawberries. Drenching strawberry plants infested with Pratylenchus penetrans with butyric acid (0.1 and 1 M) reduced nematode densities by 98 – 100 %. The results of their
research prove the hypothesis that butyric acid is an alternative to synthetic substances.

Sahebani et al. (2011) in their research mentioned the impact of β-aminobutyric acid on nematodes of gherkin roots (Meloidogyne javanica). They presume that this acid is capable of improving protection reactions in gherkin roots.

Sources from the literature contain a large amount of information on the impact of the studied substances on nematode parasites of mammals, insects, and plants. Mosieni et al. (2016) observed salicylic acid to demonstrate inhibitory effects against Meloidogyne javanica, a nematode of plants, by inhibiting its reproduction in tomato plants. Nematocidal activity of acetic acid was determined by Kim et al. (2016) in the course of a study of its impact on Meloidogyne incognita, a parasite of agricultural plants. Our results were negative regarding the mortality of nematode larvae exposed to kojic acid. However, in their experiments, Kim et al. (2016) determined a much higher concentration of kojic acid for eliminating nematodes compared to the concentration we used in our experiment. Accordingly, the researchers observed death of 60% of nematodes at concentration of 333.3 mg/ml. By contrast, in our experiments, concentrations higher than 10 g/l were not used. However, even such a concentration led to similar results for its effect on non-invasive larvae (around 40% of larvae died).

One of the most widely used substances against parasites currently is formic acid. We also observed positive results against larvae of nematodes of animals: even 1% solution caused death of L1, L2, L3 of all studied species of nematodes. Although we found no data in the literature on the relative impact of this acid on vitality of mature nematodes, their larvae and eggs, it is often used against Acari parasites of bees (Underwood & Currie, 2003; Underwood & Currie, 2007). According to Underwood et al. (2003), even 0.08 and 0.16 mg/l doses of formic acid is efficient against the Acari Varroa destructor at a temperature of over 5 °C. Nonetheless, the highest medical efficiency was observed for a dose of 0.16 mg/l at the temperature of 35 °C. In treatment of coccidiosis, one may use a 1% solution of formic acid. We also observed positive results against larvae (Nematoda, Rhabditida) under the temperature of 35 °C. In treatment of coccidiosis, one may use a 1% solution of formic acid. We also observed positive results against larvae (Nematoda, Rhabditida) under the temperature of 35 °C.

Conclusion

Using food additives, including acids against parasites of animals and humans is one of the new directions in veterinary medicine and biology. Periodic addition to fodder of these substances with nematocidal properties can manage the intensity of helminth infection. Therefore, it is possible for farmers to maintain dairy and meat products at a high level without using anthelmintic preparations of synthetic origin, farmers can. Further experiments can lead to development of preparations containing formic and benzoic acids.

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