

Article

Nematicidal Activity of Organic Food Additives

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Abstract: Organic food additives are popular in various spheres of human activity: the food industry, agriculture, veterinary and human medicine, the chemical industry, and other sectors. Due to the relatively short time of their breakdown in the environment, their non-toxicity to various organisms in certain concentrations, and their large annual amounts of production, application, and influx into the environment, it is an important task to study their influence on ecology. To help reduce toxic pressure on the environment, we studied the influence of 32 organic food additives on larvae of the nematodes *Strongyloides papillosus*, *Haemonchus contortus*, *Muellerius capillaris*, which are parasites of the digestive tracts of ruminants. This article presents the results of in vitro studies of organic food additives (acetic, propionic, lactic, sorbic, adipic, fumaric, malic, citric, ascorbic, and dehydroacetic acids, sodium formate, calcium formate, potassium acetate, calcium acetate, sodium diacetate, calcium propanoate, sodium lactate, potassium sorbate, sodium sorbate, calcium sorbate, trisodium citrate, monosodium glutamate, sodium dehydroacetate, sodium benzoate, potassium benzoate, calcium benzoate, biphenyl, dibutylhydroxytoluene, hexamethylenetetramine, 2-phenylphenol, natamycin, and nisin) on nematode larvae at various stages of their development. Propionic acid and 2-phenylphenol, used in the food industry, exerted notable nematicidal effects on larvae of *S. papillosus*, *M. capillaris*, and *H. contortus*: 1% concentrations of the substances killed 85% of these larvae, except *H. contortus*, which were tolerant to 2-phenylphenol in 52% of cases. Other tested food additives at 1% concentrations could not kill more than 70% of invasive nematode larvae in 24 h. The research determined various degrees of toxicity of the studied substances to invasive (third age) and non-invasive (first and second age) larvae, which are related to a decrease in the feeding intensity of parasitic nematodes on the stage of third age larvae. Thus, some organic food additives, introduced into the environment with food wastes, are able to affect the vitality of certain nematode species to a varying extent.

Keywords: mortality of nematode larvae; *Strongyloides papillosus*; *Haemonchus contortus*; *Muellerius capillaris*; food additives



Citation: Boyko, O.; Brygadyrenko, V. Nematicidal Activity of Organic Food Additives. *Diversity* **2022**, *14*, 615. <https://doi.org/10.3390/d14080615>

Academic Editor: Jesús Fernando Ayala-Zavala

Received: 20 June 2022

Accepted: 28 July 2022

Published: 30 July 2022

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1. Introduction

Food additives play a significant role in the diet of modern people because most ready-to-use foods available in stores (products of advanced and highly advanced processing) contain food additives [1]. The greater the processing of the product, and the higher the temperature at which it is stored, the more its content of preservatives, stabilizers, emulsifiers, and other substances that prolong its lifetime on the market. Therefore, many products contain 3–5% rather than 1% of food additives, in relation to their mass [1,2]. The world's population consumes food additives in varying amounts: people with a high income consume fewer additives, because they are aware of the harm many of those substances cause to health; the poorest portion of the Earth's population and people living in rural area consume relatively small quantities of additives. The greatest quantities of

additives are consumed by city residents in countries having low and average levels of income per capita.

Produced food additives, often in unused products that are thrown away, end up in landfills of solid municipal wastes: in rich countries, food waste accounts for 20–25% of the total amount of food; in poor countries, it accounts for 10% of food. Thus, at least 10% of additives (i.e., 2.81 M T per year) are introduced into the environment unchanged. In addition, some additives consumed by people, after passing through the intestine, enter sewage systems unaltered (and, in some of the poorest countries, directly into the environment without any filtrating system). These food additives create a constant source of contamination in territories around settlements [2–6]. The greater portion of organic substances is broken down quickly by soil microorganisms [7], but some food additives are able to influence fauna of natural ecosystems [8]. Therefore, the effects of various concentrations of organic food additives on different groups of soil invertebrates have become a subject of interest.

The influence of food additives has only been studied for separate groups of invertebrates: Acari [9,10], insects [11–15], and nematodes [16–19]. In some gradients of their concentration, food additives are able to cause migration of certain invertebrates [20], alter the intensity of metabolic processes in living organisms [21], or cause their death [14,15].

A significant proportion of food additives are totally safe for natural ecosystems, because their molecules enter biochemical processes in cytoplasm and mitochondria of cells: for example, acetic acids, lactic acid, citric acid, ascorbic acid, potassium acetate, calcium acetate, and sodium diacetate. Other additives, for example, biphenyl, 2-phenylphenol, hexamethylenetetramine, are absent inside cells of living organisms and the environment, and therefore their influence on invertebrates is of great interest from an ecological perspective, and in the study of possibilities for regulating certain groups of organisms that are dangerous for humans and agriculture [2]. To date, the scientific literature has accumulated a large amount of data on the properties of organic food additives and their application in the food industry. However, we are faced with a lack of information about the effect of these substances on different groups of living organisms. Such studies are innovative in the field of pest control of farm animals. Experiments on the effects of food additives on nematodes provide an opportunity to create alternative methods in relation to classical therapeutic and prophylactic measures in veterinary medicine. The objectives of this article were to examine the effects of broadly used organic food additives on the vitality of nematode larvae, which are parasites of farm mammals, in laboratory conditions.

2. Materials and Methods

Feces of ruminants were collected in the territory of the clinical-diagnostic center of the Dnipro National Agrarian-Economic University (Dnipropetrovsk Oblast, Ukraine). Goats were naturally infected (while grazing in meadows) with *Strongyloides papillosus* (Wedl, 1856), *Haemonchus contortus* (Rudolphi, 1803) and *Muellerius capillaris* (Muller, 1889). To find eggs of helminths, the samples were studied using the McMaster technique [22]. Larvae of *S. papillosus* and *H. contortus* were cultivated in feces for 10 days at the temperature of 18–22 °C. To discover larvae of *S. papillosus* and *H. contortus* and *M. capillaris*, we used the Baermann test [22]. The experiment was carried out on first and second age larvae (L1, L2) and third age larvae (L3) of *S. papillosus*, third age (L3) *H. contortus*, and first age (L1) *M. capillaris*, living in the environment. The larvae were identified based on morphological parameters [23,24]. We took into account the internal structure of the larvae: presence of intestinal cells, their number, number of rows in which the cells were arranged, shape of cells, and form of the esophagus.

After finding and identification, the larvae were placed in water (4 mL) in test tubes of 10 mL and centrifuged for 4 min at 1500 rpm. After removing the supernatant, the larvae in the vessel were uniformly stirred and placed into 1.5 mL plastic test tubes, with 0.1 mL in each. Then, solutions (1%, 0.1%, 0.01%) of the studied substances were alternately added to cultivated larvae. Distilled water was used as a control. The exposure lasted 24 h at the

temperature of 22 °C. In the process of the experiment, after exposure to the tested food additives, we determined the numbers of live and dead individuals. When confirming death of the larvae, we took into account the combination of two factors: the mobility of larvae and the breakdown of the intestinal tissue.

Larvae were subjected to the influence of organic food additives (Tables 1 and 2) in five replications for each of the variants of the experiment. For the experiment, we used acetic, propionic, lactic, sorbic, adipic, fumaric, malic, citric, ascorbic, and dehydroacetic acids, sodium formate, calcium formate, potassium acetate, calcium acetate, sodium diacetate, calcium propanoate, sodium lactate, potassium sorbate, sodium sorbate, calcium sorbate, trisodium citrate, monosodium glutamate, sodium dehydroacetate, sodium benzoate, potassium benzoate, calcium benzoate, biphenyl, dibutylhydroxytoluene, hexamethylenetetramine, 2-phenylphenol, natamycin, and nisin (Alma-Veko Food, Kyiv, Ukraine).

Table 1. Use of 32 organic food additives utilized for determining level of survivability of nematodes in the laboratory experiment.

Name	Code	Class of Additives	Commonest Uses
Acetic acid	E260	preservatives	food industry, domestic cooking, for preservation; in large amounts is used in the chemical industry, for obtaining medicinal and aroma compounds
Propionic acid	E280	preservatives	prevents growth of mold and some species of bacteria; preservative in food products and fodders for animals; in production of herbicides, drugs, aroma compounds, plastics; solvents, surfactants
Lactic acid	E270	preservatives	food industry (preservative and acidifier in beverages, confectionery, meat products, vessels) and polymers production
Sorbic acid	E200	preservatives	for prevention of development of mould in non-alcoholic beverages, juices, bakery products, marmelade, jams, cheeses, fish caviar, semi-smoked sausages, condensed milk; for processing packing materials for food products
Adipic acid	E355	antioxidants	for production of nylon and polyurethane; to provide non-alcoholic beverages with bitter taste; in substances for removal of limescale
Fumaric acid	E297	preservatives	to prepare beverages, bakery, acidifier for sweets; in medicine for treatment of psoriasis, for is formed in the skin, during exposure to sunlight
Malic acid	E296	preservatives	to prepare beverages and confectionary; provides products with sour taste, is the main acid in many fruits (apricots, blackberry, European blueberry, cherry, grapes, peaches, pears, cherry plums and quinces); used in combination with less acidic citral acid or instead of it in sour sweets
Citric acid	E330	antioxidants	in the food industry and production of beverages, processed cheeses; cosmetics; substance for removal of limescale; additive to cement and gypsum concrete in construction
Ascorbic acid	E300	antioxidants	in the food industry to prevent oxidation of products; in cosmetology for restoration of protective functions of the skin; participates in formation of collagen and synthesis of corticosteroids, decreases amount of free radicals in the organisms of humans and animals
Dehydroacetic acid	E265	preservatives	is used as fungicide and bactericidal substance for preservation of fruits and vegetables, for adhesives for packing materials; plastifier for obtaining synthetic resins, preservative in cosmetics
Sodium formate	E237	preservatives	inhibitor of corrosion in anti-icing mixtures; in the tanning industry as tannins; in textile industry for dyeing fabrics; in construction to improve frost resistance of concrete
Calcium formate	E238	preservatives	accelerator of solidification of construction mixtures and concrete, which increases its durability; preservative in non-alcoholic beverages; for pickling cabbage and other vegetables; substitute for salt in dietary products; for fish preservation; cosmetics; for tanning leather and dyeing of fabric
Potassium acetate	E261	preservatives	reagent for prevention of icing-over at airports; used for substances to extinguish fire; used intravenously for maintenance treatment of ketoacidosis; as preservative and acidity regulator in the food industry
Calcium acetate	E263	preservatives	as catalyzer to obtain lavsan, regulator of acidity and compactor of plant tissues in the food industry; in bakery products, during preservation of vegetables and fruits; in livestock breeding for preservation of fodders; to correct calcium balance in people with kidney failure
Sodium diacetate	E262	preservatives	in the food industry, to provide product with taste of vinegar and salt-specific artificial dry vinegar; during preparation of crisps, crackers and other dry products
Calcium propanoate	E282	preservatives	in industrial bakery, it prevents development of mould on bakery goods, dairy and meat products; broadly used in cosmetics; additive to animal fodders; stimulates development of obesity and induces diabetes in humans
Sodium lactate	E325	antioxidants	in production of liqueurs, creams, cocktails for increasing their storage period; to increase storage periods of frozen vacuum packed meat goods; during preservation of tomatoes, cucumbers, onion, olives; for fermentation and providing bread with characteristic taste; in compounds of shampoos, creams and other cosmetics

Table 1. Cont.

Name	Code	Class of Additives	Commonest Uses
Potassium sorbate	E202	preservatives	to inhibit growth of mold and yeasts on cheese, wine, yogurts, meat, non-alcoholic beverages, bakery; to prepare butter, margarine, mayonnaise, ketchup, smoked meat and sausages, tomato puree, jam, powidl, sweets, waffles, chocolate; to protect dry fruits from mould; in personal hygiene products, cosmetics; acidulant in animal feeders
Sodium sorbate	E201	preservatives	is added as preservative to margarine, cheese, tvorog, pâté, preserved olives and vegetables, dry fruits, syrups, jelly, bubble gum, sweets, chocolate, ready salads, caviar and shrimps, non-alcoholic beverages; unlike calcium sorbate, has potential genotoxic effects, provokes allergic reactions; the additive is prohibited in the territory of the EU
Calcium sorbate	E203	preservatives	inhibits the growth of molds and some bacteria in food products; in bakery, cheese, tvorog and other dairy products, chocolate and non-alcoholic beverages; broadly used in cosmetics; causes hives, allergic reactions, asthma; the additive is prohibited in the territory of the EU
Trisodium citrate	E331	antioxidants	as food additive is included in many gassed, energy beverages; used for regulation of acidifier of many meals; salt-melting agent in production of processed cheese; present in many instant drugs; used in coffee machines; as anticoagulant for blood storage; broadly used in glass cleaners and retards the setting of gypsum-concrete mixtures
Monosodium glutamate	E621	flavor enhancers	acidifier in many meat products, bouillon cubes, soups, condiments, sauces; imitates the taste of protein-rich products (taste of umami)
Sodium dehydroacetate	E266	preservatives	used as a fungicide, plasticizer, toothpaste, preservative in food; used in cosmetics and personal care products because of its antimicrobial properties; it appears in a variety of products, including bath, skin care, suntan lotion, sunscreen, fragrance, shaving, hair and nail care products, and eye and facial makeup, because of its ability to kill microorganisms
Sodium benzoate	E211	preservatives	fruits and vegetables can be rich sources of sodium benzoate, especially cranberry and European blueberry; is present in sea products, especially in shrimps, dairy products; inhibits development of yeasts and fungi, inhibits enzymatic activity in cells, breakdown of fat and starch; broadly used in ready salads, in gassed beverages, jams, fruit juices, salted cucumbers, condiments, filling, meat and fish products, margarine, mayonnaise, ketchup, used in fireworks and powder for whistling sound
Potassium benzoate	E212	preservatives	inhibits growth of mold, yeasts and some species of bacteria; added to fruit juices, gassed beverages, pickles; in presence of ascorbic acid in products, easily becomes toxic benzene in sweetened beverages
Calcium benzoate	E213	preservatives	in non-alcoholic beverages, fruit juices, preservatives, soybean milk, soybean sauce and vinegar; most broadly used as preservative in bakery production; as preservative in substances for mouthwashes; is in substances for decreasing water hardness
Biphenyl (diphenil)	E230	preservatives	present in coal tar and raw oil; biphenyl is insoluble in water, but soluble in organic solvents; is an intermediate product for production of many organic compounds—emulsifiers, optical bleaches, substances for protection of plants and plastics; initial material for production of extremely toxic polychlorinated biphenyls (PCBs); prevents growth of mold, broadly used in treatment of fruits of citrus fruits, apples, pineapples prior to their transportation; is prohibited as food additive in the EU
Dibutylhydroxytoluene (butylated hydroxytoluene)	E321	antioxidants	food additive that prevents change in colour, taste and texture of products; ingredient for domestic goods, industrial additive, antioxidant in hygienic products and cosmetic goods; a constituent for synthesis of pesticides; ingredient for preparation of plastics and rubbers; likely induces carcinogenesis
Hexamethylenetetramine	E239	preservatives	broadly used for production of cheese, and also preservation of caviar; has sweet taste; used for treatment of urinary tract infections, transforms into carcinogenic formaldehyde in acidic environment; antidepressant, for removing sweat smell by binding property of formaldehyde; raw material for production of explosives
2-Phenylphenol (o-phenylphenol)	E231	preservatives	agricultural fungicide; it is most often used for coating citrus fruits, apples and other fruits; disinfecting substance for surfaces and wetlands, apartments, in farms, enterprises of the food industry; used in production of other fungicides, colourings, resins and rubbers; present in sprays and deodorants for armpits; is confirmed carcinogen; forbidden as food additive in the EU
Natamycin	E235	preservatives	used for treatment of candidosis of the intestines, infections of the skin and mucous membranes, caused by <i>Candida</i> , other yeast-like fungi or dermatophytes; in food industry, it is used for prevention of growth of fungi; is applied to the surfaces of meat, sausages, cheese; is introduced into ready salads
Nisin	E234	preservatives	used in composition of processed cheese, meat, beverages for prolonging storage period by inhibiting growth of Gram-positive bacteria; broadly used for bakery preparation; is low-toxic preservative for humans

Data are generalized based on Branen et al. [25], Singh et al. [26], and also data from open sources.

Table 2. Maximum level (mg/kg) of food additives permitted for use by the Codex Alimentarius in basic human foods.

Food Additives	Meat Products	Milk and Dairy Products	Cheese	Vegetables, Fruits	Beverages	Bread, Bakery Products	Candy, Jams, Jellies, Marmalades	Baby Food
Acetic acid	–	–	GMP	vegetables GMP	GMP	–	–	5000
Ascorbic acid	GMP	GMP	–	vegetables 500, GMP	GMP	–	–	500
Lactic acid	–	–	–	GMP	–	–	–	2000
Citric acid	GMP	GMP	GMP	vegetables GMP	3000–5000, GMP	–	–	–
Fumaric acid	–	–	–	GMP	GMP	–	–	–
Malic acid	–	GMP	GMP	vegetables GMP	GMP	–	–	GMP
Propionic acid	–	–	3000, GMP	–	–	–	–	–
Potassium acetate	–	–	–	–	–	–	–	GMP
Sodium diacetate	1000	–	–	–	–	4000	1000	–
Sodium lactate	–	GMP	–	vegetables GMP	GMP	–	–	GMP
Sorbates	200–2000	1000	1000–3000	fruit 500–1000, vegetables 1000	500		1000–1500	
Calcium acetate	–	–	–	–	–	–	–	GMP
Calcium propanoate	–	–	3000, GMP	–	–	–	–	–
Hexamethylenetetramine	–	–	25	–	–	–	–	–
Monosodium glutamate	–	–	–	vegetables GMP	GMP	–	–	–
Natamycin	6–20	–	40	–	–	–	–	–
Nisin	25	12.5	12.5	–	–	–	–	–
Trisodium citrate	GMP	GMP	–	vegetables GMP	GMP	–	–	5000


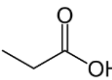

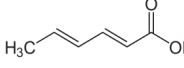
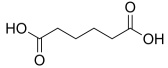
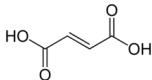
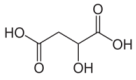
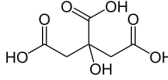
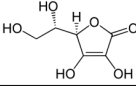
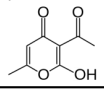
Data are generalized based on Codex Alimentarius; GMP–good manufacturing practices.

The statistical analysis of the results was performed using Statistica 12 (StatSoft Inc., Tulsa, OK, USA). Tables present average value (\bar{x}) \pm standard deviation (SD). Differences between the values of the control and experimental groups were determined using the Tukey test, where the differences were considered significant at $p < 0.05$.

3. Results

The results of the research on the influence of organic acids used in the food industry on the vitality of nematodes of ruminants (Table 3) revealed notable nematicidal properties of propionic acid. At 24 h, its 1% solution killed over 85% of larvae of the tested species of nematodes at various stages of development. At the same time, a high proportion of mortality was observed for non-invasive larvae, in addition to more-tolerant invasive larvae. The larvae that were most tolerant to 1% concentration of this acid were invasive *H. contortus*. Nonetheless, over 87.5% of these died in the 24 h experiment.

Table 3. Mortality of larvae of *S. papillosus*, *H. contortus*, and *M. capillaris* (%) during 24 h laboratory experiment under the influence of organic acids used as food additives ($x \pm SD$, $n = 5$).

Substance	Nematode Species	Mortality of Nematode Larvae in Control, %	Mortality of Nematode Larvae in 1% Solution, %	Mortality of Nematode Larvae in 0.1% Solution, %	Mortality of Nematode Larvae in 0.01% Solution, %	Lowest Effective Concentration (Mortality over 70%)
Acetic acid 	L ₁₋₂ of <i>S. papillosus</i>	16.9 \pm 7.2 ^a	94.7 \pm 6.6 ^b	52.7 \pm 5.8 ^c	18.4 \pm 4.6 ^a	1%
	L ₃ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	4.8 \pm 11.7 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₃ of <i>H. contortus</i>	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	0.0 \pm 0.0 ^a	100.0 \pm 0.0 ^b	4.9 \pm 5.4 ^a	0.0 \pm 0.0 ^a	1%
Propionic acid 	L ₁₋₂ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	100.0 \pm 0.0 ^b	30.3 \pm 3.3 ^c	7.2 \pm 4.1 ^d	1%
	L ₃ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	100.0 \pm 0.0 ^b	23.7 \pm 3.2 ^c	5.6 \pm 3.4 ^d	1%
	L ₃ of <i>H. contortus</i>	0.0 \pm 0.0 ^a	87.5 \pm 2.3 ^b	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	1%
	L ₁ of <i>M. capillaris</i>	6.7 \pm 14.9 ^a	95.3 \pm 7.5 ^b	25.8 \pm 14.2 ^a	14.5 \pm 8.4 ^a	1%
Lactic acid 	L ₁₋₂ of <i>S. papillosus</i>	17.8 \pm 7.8 ^a	81.8 \pm 9.7 ^b	49.1 \pm 7.7 ^c	23.0 \pm 9.0 ^a	1%
	L ₃ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₃ of <i>H. contortus</i>	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	0.0 \pm 0.0 ^a	87.6 \pm 2.3 ^b	2.7 \pm 4.2 ^a	0.0 \pm 0.0 ^a	1%
Sorbic acid 	L ₁₋₂ of <i>S. papillosus</i>	23.7 \pm 8.7 ^a	100.0 \pm 0.0 ^b	58.7 \pm 9.2 ^c	25.4 \pm 4.5 ^a	1%
	L ₃ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	4.0 \pm 8.9 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₃ of <i>H. contortus</i>	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	1.0 \pm 2.6 ^a	0.0 \pm 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	5.9 \pm 5.5 ^a	28.6 \pm 7.5 ^b	15.9 \pm 10.5 ^{ab}	15.4 \pm 8.8 ^{ab}	—
Adipic acid 	L ₁₋₂ of <i>S. papillosus</i>	23.7 \pm 8.7 ^a	100.0 \pm 0.0 ^b	72.3 \pm 10.4 ^c	28.0 \pm 5.5 ^a	0.1%
	L ₃ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₃ of <i>H. contortus</i>	0.0 \pm 0.0 ^a	3.8 \pm 6.1 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	0.0 \pm 0.0 ^a	100.0 \pm 0.0 ^b	21.0 \pm 10.6 ^c	0.0 \pm 0.0 ^a	1%
Fumaric acid 	L ₁₋₂ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	87.0 \pm 3.5 ^b	50.6 \pm 7.0 ^c	0.0 \pm 0.0 ^a	1%
	L ₃ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	36.1 \pm 8.7 ^b	25.2 \pm 2.4 ^b	0.0 \pm 0.0 ^a	—
	L ₃ of <i>H. contortus</i>	0.0 \pm 0.0 ^a	17.6 \pm 7.6 ^b	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	0.0 \pm 0.0 ^a	94.7 \pm 6.3 ^b	27.9 \pm 18.4 ^c	0.0 \pm 0.0 ^a	1%
Malic acid 	L ₁₋₂ of <i>S. papillosus</i>	17.6 \pm 7.8 ^a	90.1 \pm 9.0 ^b	47.0 \pm 7.3 ^c	24.0 \pm 2.0 ^a	1%
	L ₃ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	5.7 \pm 9.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₃ of <i>H. contortus</i>	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	0.0 \pm 0.0 ^a	24.9 \pm 20.5 ^b	1.5 \pm 2.7 ^{ab}	2.3 \pm 3.0 ^{ab}	—
Citric acid 	L ₁₋₂ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	100.0 \pm 0.0 ^b	44.2 \pm 4.3 ^c	0.0 \pm 0.0 ^a	1%
	L ₃ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	27.4 \pm 3.3 ^b	17.6 \pm 5.7 ^b	0.0 \pm 0.0 ^a	—
	L ₃ of <i>H. contortus</i>	0.0 \pm 0.0 ^a	11.1 \pm 2.3 ^b	2.8 \pm 5.6 ^{ab}	0.0 \pm 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	0.0 \pm 0.0 ^a	96.0 \pm 8.9 ^b	14.7 \pm 13.8 ^c	0.0 \pm 0.0 ^a	1%
Ascorbic acid 	L ₁₋₂ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	94.2 \pm 8.1 ^b	30.6 \pm 22.3 ^c	0.0 \pm 0.0 ^a	1%
	L ₃ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	63.5 \pm 6.3 ^b	31.0 \pm 7.4 ^c	0.0 \pm 0.0 ^a	—
	L ₃ of <i>H. contortus</i>	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	0.0 \pm 0.0 ^a	92.7 \pm 10.1 ^b	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	1%
Dehydroacetic acid 	L ₁₋₂ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	55.1 \pm 3.1 ^b	39.6 \pm 4.9 ^c	0.0 \pm 0.0 ^a	—
	L ₃ of <i>S. papillosus</i>	0.0 \pm 0.0 ^a	18.2 \pm 1.7 ^b	12.4 \pm 6.5 ^b	0.0 \pm 0.0 ^a	—
	L ₃ of <i>H. contortus</i>	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	0.0 \pm 0.0 ^a	10.0 \pm 14.9 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	—

Different letters in Table within each line indicate significant ($p < 0.05$) differences between groups according to Tukey's test results.

Other acids exerted various effects on the vitality of larvae of nematode species at different stages of their development. Therefore, acetic, adipic, lactic, fumaric, citric, and ascorbic acids had the strongest effects on *S. papillosus* larvae of the first and second stages and *M. capillaris* of the first stage. Invasive larvae of *S. papillosus* and *H. contortus* were most tolerant to those acids. Sorbic and malic acids displayed actions only toward non-invasive stages of *S. papillosus*.


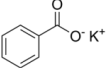
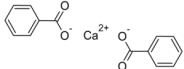
In general, the study of the influence of salts of organic acids on the vitality of nematode larvae of ruminants revealed a smaller effect compared with the acids (Table 4). Third-stage larvae of *H. contortus* remained vital for 24 h or longer in more than 85% of cases. At the same time, no damaged intestinal cells were found in nematodes. Invasive larvae of *S. papillosus* were also affected by salts of organic acids, but no more often than in 40% cases (calcium propanoate). During the 24 h period, most tested salts of organic acids in 1%

concentration, and also their weaker solutions, had no significant effect on the vitality of invasive nematode larvae.

Table 4. Mortality of larvae of *S. papillosus*, *H. contortus*, and *M. capillaris* (%) during 24 h laboratory experiment under the influence of salts of organic acids, used as food additives ($x \pm SD$, $n = 5$).

Substance	Nematode Species	Mortality of Nematode Larvae in Control, %	Mortality of Nematode Larvae in 1% Solution, %	Mortality of Nematode Larvae in 0.1% Solution, %	Mortality of Nematode Larvae in 0.01% Solution, %	Lowest Effective Concentration (Mortality over 70%)
Sodium formate $\left[\begin{array}{c} \text{O} \\ \parallel \\ \text{H}-\text{C}-\text{O}^- \\ \\ \text{O} \end{array} \right] \text{Na}^+$	L _{1–2} of <i>S. papillosus</i>	11.4 ± 1.5 ^a	67.1 ± 3.6 ^b	31.9 ± 2.8 ^c	10.9 ± 6.3 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	36.8 ± 2.4 ^b	18.7 ± 1.3 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	57.9 ± 23.5 ^b	7.7 ± 7.1 ^c	6.2 ± 8.5 ^{ac}	–
Calcium formate $\left[\begin{array}{c} \text{O} \\ \parallel \\ \text{H}-\text{C}-\text{O}^- \\ \\ \text{O} \end{array} \right]_2 \text{Ca}^{2+}$	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	16.3 ± 2.3 ^b	6.6 ± 1.9 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	2.0 ± 2.4 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	2.9 ± 6.4 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	37.4 ± 8.6 ^b	14.7 ± 10.4 ^c	6.0 ± 8.9 ^{ac}	–
Potassium acetate $\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{C}-\text{O}^- \text{K}^+ \end{array}$	L _{1–2} of <i>S. papillosus</i>	19.6 ± 1.4 ^a	55.8 ± 5.1 ^b	37.4 ± 1.6 ^c	18.9 ± 4.5 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	19.7 ± 2.9 ^b	9.8 ± 1.5 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	35.1 ± 21.9 ^b	24.4 ± 9.9 ^b	19.9 ± 13.5 ^b	–
Calcium acetate $\left[\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{C}-\text{O}^- \end{array} \right]_2 \text{Ca}^{2+}$	L _{1–2} of <i>S. papillosus</i>	19.6 ± 1.4 ^a	34.0 ± 4.6 ^b	20.2 ± 3.3 ^a	19.3 ± 6.2 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	7.5 ± 2.0 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	4.0 ± 8.9 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	38.7 ± 9.5 ^b	27.8 ± 18.8 ^b	6.7 ± 9.1 ^{ab}	–
Sodium diacetate $\text{Na}^+ \left[\begin{array}{c} \text{H}_3\text{C}-\text{C}(=\text{O})-\text{O}^- \\ \\ \text{H} \\ \\ \text{H}_3\text{C}-\text{C}(=\text{O})-\text{O}^- \end{array} \right]$	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	60.4 ± 7.4 ^b	39.6 ± 2.4 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	33.2 ± 8.4 ^b	23.8 ± 1.4 ^b	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
Calcium propanoate $\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{CH}_2-\text{C}-\text{O}^- \end{array} \text{Ca}^{2+}$	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	45.1 ± 4.3 ^b	39.1 ± 6.4 ^b	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	43.9 ± 5.0 ^b	11.5 ± 3.6 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	14.6 ± 2.4 ^b	5.6 ± 6.6 ^{ab}	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	90.0 ± 22.4 ^b	28.7 ± 19.4 ^c	0.0 ± 0.0 ^a	1%
Sodium lactate $\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{CH}-\text{C}-\text{O}^- \text{Na}^+ \\ \\ \text{OH} \end{array}$	L _{1–2} of <i>S. papillosus</i>	18.9 ± 5.7 ^a	37.9 ± 10.4 ^b	17.6 ± 2.5 ^a	16.2 ± 3.1 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
Potassium sorbate $\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{C}-\text{O}^- \text{K}^+ \end{array}$	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	36.1 ± 3.6 ^b	33.8 ± 4.0 ^b	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	18.7 ± 6.2 ^b	21.7 ± 1.3 ^b	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
Sodium sorbate $\text{H}_3\text{C}-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{C}(=\text{O})\text{O}^- \text{Na}^+$	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	42.3 ± 3.4 ^b	18.2 ± 2.5 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	17.9 ± 0.6 ^b	10.1 ± 1.6 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	7.9 ± 11.4 ^a	2.9 ± 6.4 ^a	2.0 ± 4.5 ^a	–
Calcium sorbate $\left[\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{C}-\text{O}^- \end{array} \right]_2 \text{Ca}^{2+}$	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	20.0 ± 1.7 ^b	14.8 ± 1.6 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	10.0 ± 3.5 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
Trisodium citrate $\text{Na}^+ \text{O}^- \text{C}(=\text{O})\text{CH}(\text{OH})\text{C}(=\text{O})\text{O}^- \text{Na}^+ \text{O}^- \text{C}(=\text{O})\text{CH}_2\text{COO}^- \text{Na}^+$	L _{1–2} of <i>S. papillosus</i>	15.3 ± 5.6 ^a	15.3 ± 10.0 ^a	22.7 ± 6.8 ^a	17.3 ± 8.2 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	1.5 ± 3.7 ^a	1.4 ± 2.3 ^a	0.8 ± 1.9 ^a	–
Monosodium glutamate $\text{HOOC}-\text{CH}_2-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COO}^- \text{Na}^+$	L _{1–2} of <i>S. papillosus</i>	14.2 ± 5.9 ^a	20.1 ± 7.8 ^a	19.8 ± 8.8 ^a	14.2 ± 3.5 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	16.3 ± 3.9 ^a	20.9 ± 5.7 ^a	19.9 ± 6.4 ^a	17.2 ± 3.5 ^a	–
Sodium dehydroacetate $\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{C}-\text{O}^- \text{Na}^+ \end{array}$	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	34.9 ± 3.2 ^b	27.8 ± 3.1 ^b	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	25.5 ± 3.3 ^b	14.4 ± 1.5 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–

Table 4. Cont.

Substance	Nematode Species	Mortality of Nematode Larvae in Control, %	Mortality of Nematode Larvae in 1% Solution, %	Mortality of Nematode Larvae in 0.1% Solution, %	Mortality of Nematode Larvae in 0.01% Solution, %	Lowest Effective Concentration (Mortality over 70%)
Sodium benzoate 	L _{1–2} of <i>S. papillosus</i>	11.6 ± 7.0 ^a	19.4 ± 4.7 ^a	17.8 ± 5.1 ^a	17.1 ± 6.6 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	19.5 ± 8.0 ^a	22.3 ± 11.8 ^a	21.5 ± 12.7 ^a	21.1 ± 7.6 ^a	–
Potassium benzoate 	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	33.6 ± 3.7 ^b	24.6 ± 2.5 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	32.0 ± 4.0 ^b	16.9 ± 2.8 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
Calcium benzoate 	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	37.7 ± 2.3 ^b	22.7 ± 2.6 ^c	9.2 ± 1.2 ^d	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	19.5 ± 1.6 ^b	11.4 ± 1.5 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	14.8 ± 3.8 ^b	4.2 ± 3.9 ^a	0.0 ± 0.0 ^a	–

Different letters in Table within each line indicate significant ($p < 0.05$) differences between groups according to Tukey's test results.

Less tolerant to the exposure to salts of organic salts were non-invasive stages of nematode larvae. Therefore, under 24 h of the influence of 1% solution of calcium propanoate, no more than 10% of first-stage larvae of *M. capillaris* remained vital. Sodium formate also had a significant effect on *M. capillaris*. Over 50% of first-stage larvae of this nematode species died in 1% solution of this food additive. Moreover, sodium formate had an effect on non-invasive stages of *S. papillosus* larvae. *S. papillosus* of this age were also affected by potassium acetate and sodium diacetate (more than 50% of them died during 24 h exposure to 1% solutions).

Research on the influence of salts of organic food additives of other groups on nematode larvae of ruminants revealed a significant effect of 2-phenylphenol (Table 5). Twenty-four-hour exposure to its 1% solution was lethal for all larvae of *S. papillosus* and *M. capillaris*. The most tolerant larvae to this substance were invasive *H. contortus*. Despite the fact that the proportion of vital third-stage larvae of this species was 51%, 0.1% concentration of 2-phenylphenol also significantly affected the first- and second-stage *S. papillosus*, and also first-stage *M. capillaris*. Over 55% of these larvae died in 24 h under the influence of 0.1% concentration of 2-phenylphenol. However, third-stage larvae of *S. papillosus* and *H. contortus* were more tolerant to this concentration of 2-phenylphenol. Biphenyl and hexamethylenetetramine in 1% concentrations caused death to more than 50% of first- and second-stage larvae of *S. papillosus*. The remaining tested food additives in Table 4 had no significant effects on either invasive or non-invasive nematode larvae.

Table 5. Mortality of larvae of *S. papillosus*, *H. contortus*, and *M. capillaris* (%) during 24 h laboratory experiment under the influence of salts of organic food additives of various groups ($x \pm SD$, $n = 5$).

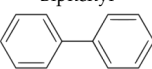
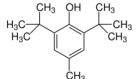

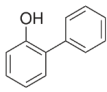
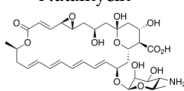
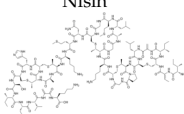
Substance	Nematode Species	Mortality of Nematode Larvae in Control, %	Mortality of Nematode Larvae in 1% Solution, %	Mortality of Nematode Larvae in 0.1% Solution, %	Mortality of Nematode Larvae in 0.01% Solution, %	Lowest Effective Concentration (Mortality over 70%)
Biphenyl 	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	51.1 ± 1.7 ^b	32.4 ± 1.4 ^c	10.1 ± 1.4 ^d	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	29.9 ± 2.3 ^b	12.8 ± 2.2 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
Dibutylhydroxytoluene 	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	21.9 ± 1.3 ^b	13.5 ± 3.2 ^c	7.7 ± 2.4 ^c	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	4.1 ± 1.5 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	4.0 ± 8.9 ^a	0.0 ± 0.0 ^a	3.3 ± 7.5 ^a	–
Hexamethylenetetramine 	L _{1–2} of <i>S. papillosus</i>	11.4 ± 1.5 ^a	55.6 ± 2.0 ^b	37.8 ± 1.6 ^c	12.7 ± 4.6 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	14.7 ± 1.9 ^b	11.4 ± 1.6 ^b	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	13.6 ± 13.9 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–

Table 5. Cont.

Substance	Nematode Species	Mortality of Nematode Larvae in Control, %	Mortality of Nematode Larvae in 1% Solution, %	Mortality of Nematode Larvae in 0.1% Solution, %	Mortality of Nematode Larvae in 0.01% Solution, %	Lowest Effective Concentration (Mortality over 70%)
 2-Phenylphenol	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	100.0 ± 0.0 ^b	55.0 ± 4.8 ^c	0.0 ± 0.0 ^a	1%
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	100.0 ± 0.0 ^b	34.1 ± 2.3 ^c	0.0 ± 0.0 ^a	1%
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	48.7 ± 40.2 ^{ab}	14.0 ± 21.9 ^a	7.6 ± 10.5 ^a	–
	L ₁ of <i>M. capillaris</i>	2.9 ± 6.4 ^a	100.0 ± 0.0 ^b	66.0 ± 32.6 ^c	8.9 ± 14.5 ^a	1%
 Natamycin	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	18.3 ± 2.2 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	16.5 ± 1.3 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
 Nisin	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	29.8 ± 3.7 ^b	24.0 ± 2.3 ^b	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	10.8 ± 3.5 ^b	8.5 ± 4.1 ^b	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–

Different letters in Table within each line indicate significant ($p < 0.05$) differences between groups according to Tukey's test results.

4. Discussion

Studies into combating parasites of agricultural animals deal not only with influence of nematicidal drugs, but also substances used in the food industry, and plants that may be used as food additives for agricultural animals. Our previous studies [27,28] were focused on effects on medicinal plants on the vitality of nematodes in the environment.

Organic food additives are used not only in the food industry, they are also universal ingredients used in pharmaceuticals and perfumery as solvents, and in the oil and textile industries. These substances are constituents of detergents, plastics, rubbers, colorings, and glues [29], and they are broadly used in the production of chemical substances in the automobile and construction industries. Over the last few years, there have been many experimental studies of the use of organic food additives in agriculture for fighting pests. This direction is especially popular in organic arable farming and animal husbandry, where drugs and other chemically synthesized substances are prohibited. Many farms are currently reducing the amounts of pesticides they use in order to decrease toxic pressure on natural ecosystems. Their negative impact on ecosystems manifests through accumulation in the environment, because they do not break down easily and can remain in the soil for a long time. Thus, pesticides are harmful to the health of animals and people, and also have negative effects on soil microorganisms. The term “biopesticides”, referring to substances present in nature, is being used increasingly often. These are also called natural pesticides. This category includes components of some plants, bacteria, fungi, and other living organisms. They may also be substances obtained from natural sources [30,31]. Santos et al. [32] described the properties of *Bacillus thuringiensis*, which is used in the compound of bioinsecticides. This species of bacteria produces toxins and spores, which are toxic for some species of insects. The efficacy of those bioinsecticides depends on the quality of their application. Good results have been shown by the combined use of bioinsecticides and some propionic acid-based adjuvants.

Some organic food substances are also biopesticides. The Ukrainian Certifying Body “Organic Standard” is the leader in organic certification in Ukraine, and some other international certifying bodies (bio.inspecta, CERES, Control Union and others) give certificates in Ukraine, based on the law of the European Union on Organic Agriculture. The list of substances that are allowed for use as food additives for animals contains such organic acids as sorbic, formic, acetic, propionic, and citric acids. Some of these acids (formic, acetic, and oxalic) are allowed as products for disinfection. There are numerous data about the use of formic acid as a biocarbicide to combat the honey bee parasite *Varroa destructor* [33–35]. Laboratory experiments aimed at evaluation of the efficiency of various concentrations of formic acid against Acari in different temperature conditions were conducted by Underwood

and Currie [33,34]. Our previous studies of this food additive also indicated nematicidal properties of formic acid against nematode larvae, which are parasites of ruminants. The lowest LD₅₀ values were observed for formic acid: this parameter equaled 4.7 g/kg on average for third-stage larvae of *S. papillosus*, 0.076 g/kg for first- and second-stage larvae of *S. papillosus*, and 4.1 g/kg for third-stage larvae of *H. contortus* [36].

Beyhan et al. [37] evaluated the influence of acetic acid on eggs of *Ascaris lumbricoides*, which are nematodes of the digestive tract of humans: 1% solution of acetic acid caused no death to the ascarids; however, after increasing the concentration to 3%, 95% of the nematodes died. The influence of 5% acetic acid resulted in the death of all the eggs of this nematode species. One of the studies [38] was focused on the influence of short-chained and long-chained fatty acids on the vitality of eggs of *Ascaris suum*, which are parasites of the digestive tract of swine. The main objective of these studies was to determine a safe and effective method of inactivation of eggs of parasites in wastewater, without causing a negative impact on the environment. Complete inactivation (100%) of eggs of *A. suum* by acetic acid was observed for mixtures of acetic acid (288 mM), butyric acid (240 mM), and hexanoic acid (16 mM); and acetic acid (288 mM), butyric acid (240 mM), valeric acid (16 mM), and hexanoic acid (16 mM) [38]. Acetic acid is also used to dehydrate stained helminths [39]. Rojas-Oropeza et al. [40] studied the influence of organic acids used in the food industry on the vitality of eggs of *A. suum*, finding a mixture of acetic, propionic, butyric, valeric, and isovaleric acids to be effective. We also observed the effect of acetic and propionic acids on the vitality of nematode larvae of ruminants. The larvae most susceptible to acetic acids were non-invasive *S. papillosus* and *M. capillaris*. These acids may be more effective against invasive larvae in mixtures rather than the pure substances analyzed in the article. We obtained these results after exposing nematodes to lactic, ascorbic, adipic, and citric acids. However, the greatest impact on the vitality of nematode larvae of various stages was caused by 1% solution of propionic acid.

As an insecticide and fungicide, Dunkel [41] proposed using sorbic acid. It has notable insecticidal and fungicidal properties. This acid was registered in the Agency of Environmental Protection (USA) as a safe substance for maintenance of grain and maize. The concentration needed to effectively combat fungi was equal to 0.3%, whereas against some insects it was 0.5%. Furthermore, an important factor is the insect species, because a difference in concentration of even 0.1% may decrease insecticidal influence. No notable nematicidal properties of sorbic acid against nematode larvae of ruminants were found. Even in 1% concentration, over 95% of invasive larvae remained vital at 24 h. This concentration of sorbic acid only produced 100% mortality of non-invasive larvae of *S. papillosus*.

The scientific literature often contains data about natamycin, which is the food additive preservative E235. This substance is obtained using *Streptomyces natalensis* bacteria. It has notable fungicidal properties [42,43]. However, according to the results of studies, natamycin is not active against nematode larvae, which are parasites of agricultural animals. Over 80% of first- and third-stage larvae of the studied species of nematodes remained vital and mobile after 24 h of exposure.

Polychlorinated biphenyls are chemical substances that are stable in the environment. They can exert various toxic effects on living organisms and have high tolerance to bioaccumulation [44]. As our experiment on nematode larvae in the environment demonstrated, biphenyl, unbound to chlorine and used as a food additive, varies in effect on different species of parasites. The lowest tolerance to biphenyl was observed for third-stage larvae of *S. papillosus*, whereas less than 30% of invasive larvae died. Non-invasive stages of larvae of this species of nematodes were found to be less tolerant; we observed death of over 50% of larvae.

5. Conclusions

The food industry is not the only sphere in which the organic substances are valuable. Some of these substances are now used to combat agricultural pests. According to the results of our studies, the food additives propionic acid and 2-phenylphenol also have

nematocidal properties against first- and second-stage larvae of *S. papillosus*, first-stage *M. capillaris*, and invasive larvae of *S. papillosus* and *H. contortus*. Invasive larvae of *S. papillosus* were also found to be affected by sodium formate, sodium diacetate, potassium benzoate, sodium dehydroacetate, and calcium propanoate, but no more often than in 45% of cases. Non-invasive larvae, with the exception of the substances indicated above, were also susceptible to acetic, adipic, lactic, fumaric, citric, and ascorbic acids, calcium propanoate, and potassium acetate.

Food additives that are introduced into the environment in relatively small concentrations can have local negative effects on nematodes living in the soil (including free-living larval stages of nematodes, which are parasites of agricultural animals and humans). By comparison, the relatively well-studied safety of food additives for humans suggests that the potentials of these substances are not fully realized against parasitic nematodes in farms, in the disinfection of places where animals are maintained, in addition to tools and equipment used in farming.

Author Contributions: Conceptualization, O.B. and V.B.; methodology, O.B.; validation, V.B.; formal analysis, V.B.; investigation, O.B.; resources, O.B. and V.B.; data curation, O.B. and V.B.; writing—original draft preparation, O.B. and V.B.; writing—review and editing, O.B. and V.B.; visualization, O.B. and V.B.; supervision, O.B. and V.B.; project administration, O.B.; funding acquisition, O.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of Education and Science of Ukraine, grant number 0120U102384 “Ecological evaluation of use of food and fodder additives in animal husbandry”.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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