

Article

Nematicidal Activity of Inorganic Food Additives

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Abstract: The food industry is not the only sphere of human activity where inorganic food additives are globally used. In certain concentrations, they are safe for people and agricultural animals. Nonetheless, they impose a negative impact on other classes of living organisms. Therefore, our objective was to determine the influence of some inorganic food additives (alkalis, acids, salts) on the vitality of nematode larvae that parasitize agricultural animals: *Strongyloides papillosus*, *Haemonchus contortus* and *Muellerius capillaris*. We studied the effects of sodium hydroxide, potassium hydroxide, boric acid, phosphoric acid, potassium chloride, calcium chloride, sodium nitrite, potassium nitrite, sodium nitrate, potassium nitrate, ammonium bicarbonate, sodium bisulfite, sodium bisulfate, sodium sulfate, potassium sulfate, calcium sulfate, sodium thiosulfate, sodium metabisulfite, potassium metabisulfite, copper sulfate pentahydrate, tetrasodium pyrophosphate, sodium triphosphate, sodium borate decahydrate and talc. In in vitro experiments, the strongest effects were produced by alkalis sodium hydroxide and potassium hydroxide. In 24 h, 1% solutions of those substances killed 69% of larvae of *S. papillosus*, *H. contortus* and *M. capillaris* of various development stages. Sodium sulfate was effective against all stages of larvae of *S. papillosus*, and also against first-age *M. capillaris*. Nematocidal properties only against all stages of *S. papillosus* were exerted by copper sulfate pentahydrate. Non-invasive stages of *S. papillosus* nematodes were affected only by phosphoric acid, ammonium bicarbonate, calcium chloride, sodium nitrite, calcium sulfate, potassium metabisulfite, tetrasodium pyrophosphate, sodium triphosphate and the same stages of *M. capillaris*—by phosphoric acid, sodium bisulfite and potassium nitrite.

Keywords: mortality of nematode larvae; *Strongyloides papillosus*; *Haemonchus contortus*; *Muellerius capillaris*; food emulsifiers; food preservatives; food stabilizers; food thickeners; food antioxidants



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1. Introduction

Nematodes are common in agricultural and wild animals and humans, parasitizing the intestine [1], lungs [2], liver and other organs [3]. Therefore, the literature has large amounts of data regarding the struggle against the diseases those larvae cause [4,5]. The significance of those human and mammal parasites is hard to exaggerate, for many species are common in local populations of hosts, and broad use of anti-nematode drugs often leads to the spread of drug resistance among many nematode species [6,7]. The necessity of evaluation of effects of various natural [8,9] and synthesized [10,11] chemical substances on nematode larvae is of great interest in terms of ecotoxicology and evaluation of the potential of their anti-parasitic activity.

Strongyloidiasis is common in people in many countries with tropical and subtropical climates: 100–200 M people in 70 countries of the world are suffering from strongyloidiasis, including inhabitants and immigrants in countries of Southern, Eastern and Central Europe, inhabitants of the Appalachian region of the US and travelers returning from southern countries [12]. Opportunistic disseminated strongyloidiasis is usually found in patients with weakened immunity. Free-living generations of nematodes of *Strongyloides* genus

form local hotbeds of infection in soil. From eggs they lay in soil, rhabdite-like larvae (L₁₋₂) develop. In unfavourable conditions, they transform into more resistant filariform larvae (L₃), able to invade people and other mammals. They actively penetrate through the skin into the blood circulatory system or directly enter the digestive tract of vertebrates with food or water [13–15]. In the small intestine of mammals (in the duodenum and jejunum in people), they are able to reproduce for a long time (decades), forming more and more generations. Imbalance between the activity of eosinophils and threadworms causes serious health issues. By penetrating into the blood circulatory system, brain, cardiac muscle, lungs, kidneys, lymph nodes and other organs, nematodes cause inflammations in those organs. Strongyloidiasis is a serious problem in healthcare and veterinary medicine [16]. In Europe, 22 species of *Strongyloides* genus are distributed, including *S. papillosus* (Wedl, 1856), found in cattle, pigs, sheep, goats, rabbits, polecats, and rats [17–19].

Haemonchosis is spread throughout the world. Its pathogen is barber's pole worm *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898. People are rarely infected by this parasite, which mainly affects ruminants, severely harming the intestine, nervous, endocrine systems and other organs [20]. Most often, mature parasites attach to the mucous membrane of the abomasums of sheep and goats, and consume blood, causing edemas and gradual development of anemia. The disease is much more common during the years with a large amount of precipitations. Haemonchosis imposes great losses on farmers across the planet [21,22]. Over recent years, barber's pole worms have been observed to become drug-resistant. As a means of fighting this parasite, copper oxide (2 g of copper oxide in gel capsules per one goat or sheep) has begun to be introduced, but it often does not kill helminths, and only decreases the number of eggs (by 82.5% for sheep and 80.5% for goats) which are discharged into the environment [23]. The search for alternative anti-haemonchosis drugs may be very practically significant [24,25].

Muellerius capillaris (Mueller, 1889), also known as goat lungworm, belongs to the most economically harmful parasite of ruminants. Sheep and goats accidentally swallow terrestrial gastropods *Trochoidea* spp., *Helix* spp., *Theba* spp., *Abida* spp., *Zebrina* spp., *Limax* spp., *Agriolimax* spp. and other genera of Gastropoda class, Stylommatophora order, and become infected by larvae of this species of nematodes [26]. In the organisms of ruminants, they migrate through the blood circulatory system from the intestine to the subpleural space of the lungs [27]. The lung tissues of dead goats and sheep are found to hold inflammation hotbeds with white 3 cm-long filariform nematodes, accumulations of eggs, often concentrated in up to 2 cm-diameter nodes with white pus. Usually, first-age larvae hatch in the bronchi and the trachea, are swallowed by sheep and goats, travel through the intestine and are discharged into the environment with feces [28]. No publications about drug resistance of this species of nematodes have emerged yet.

Inorganic food additives are broadly used in the food industry and veterinary medicine. They include some that are highly dangerous to living organisms, including nematodes, in relatively low concentrations [29–31].

Therefore, prior to the beginning of the study, we formulated three hypotheses. The first of our hypotheses was that inorganic food additives would in general have a lesser effect on nematode larvae in soil than the organic, since most of the studied inorganic substances we studied are present in soil in some concentration, and nematodes—in the processes of their long evolution—have encountered them.

The second hypothesis could be formulated as follows: inorganic substances that do not occur in such types of soils where nematode larvae live would kill nematodes depending on the extent of their solubility in water. The third hypothesis was that the pH of the environment would have a low effect on the vitality of nematode larvae, since it is common knowledge that nematodes are one of the most successful groups of organisms living in both acidic and alkaline environments, and in ruminants' feces, nematode larvae can end up in wetland soil with pH of around 5 or solonchaks with pH of around 10.

The objective of this article was in vitro evaluation of the effects inorganic food additives take on the survivability of nematode larvae parasitizing farm animals.

2. Materials and Methods

In the experiment, we studied the influence of inorganic food additives: alkalis (sodium hydroxide, potassium hydroxide), acids (boric acid, phosphoric acid) and salts (potassium chloride, calcium chloride, sodium nitrite, potassium nitrite, sodium nitrate, potassium nitrate, ammonium bicarbonate, sodium bisulfite, sodium bisulfate, sodium sulfate, potassium sulfate, calcium sulfate, sodium thiosulfate, sodium metabisulfite, potassium metabisulfite, copper sulfate pentahydrate, tetrasodium pyrophosphate, sodium triphosphate, sodium borate decahydrate, talc) on nematode larvae of *Strongyloides papilliformis*, *Haemonchus contortus* and *Muellerius capillaris* (Table 1).

Table 1. Uses of inorganic food additives, utilized to determine the survivability of nematodes in the laboratory experiment.

Substance	Code	Class of Additives	Commonest Uses
Sodium hydroxide (NaOH)	E524	stabilizers, emulsifiers	In the food industry—an additive used to prepare caramel, cocoa, chocolate, bakery products; in the chemical industry—in production of soap, glycerol; petroleum; the refining industry—catalizer; to obtain biodiesel fuel; production of synthesized fibers, film materials; pulp and paper industry; production of aluminium, zinc and titan out of recycled material; production of surfactants of various types; in the textile industry—for mercerization and bleaching of plant fibers; refining of vegetable oils; to clean the air from harmful gases; filtration of wastewaters.
Potassium hydroxide (KOH)	E525	stabilizers, emulsifiers	In the food industry; as electrolyte in alkaline accumulators; to obtain liquid soap; for mercerization of wood cellulose in the process of making viscous fibers and threads; for processing cotton fabrics in order to increase hygroscopicity; as absorbent of “acidic” gases; as drying agent for gases that interact with KOH, as drying agent for liquids in organic chemistry; as anti-foaming agent in paper production; is included in domestic substances for washing dishes made of stainless steel; as catalizer in biodiesel production
Boric acid (H ₃ BO ₃)	E284	preservatives	In the food industry, in production of beer, meat products, products of fish processing and oil-fat complex; in pharmaceuticals as medicinal and disinfecting agent; in nuclear reactors—soluble neutron absorber; as boric plant fertilizer
Phosphoric acid (H ₃ PO ₄)	E338	antioxidants	In the food industry to acidify foods, beverages, as preservative and antioxidant; for production of fertilizers; for corrosion inhibition and removal of rust from metal surfaces; phosphate coating of steel, titan, nickel and their alloys; chemical milling of ferrous metals; production of fireproof materials; to create phosphoric acid fuel elements; production of activated carbon; disinfecting substance in the dairy, food industry and breweries; pH regulator in cosmetics and means of hygiene
Potassium chloride (KCl)	E508	stabilizers, emulsifiers	In the food industry; raw material for cosmetics; broadly used in agriculture as potassium fertilizer; is a raw material for obtaining potassium hydroxide and other potassium compounds
Calcium chloride (CaCl ₂)	E509	stabilizers, emulsifiers	In the food industry, it is added as thickener into dry products: milk, food mixtures, as anti-cracking agent, for mineral enrichment; in production of fermentation products, calcium chloride promotes fast milk coagulation, improves quality of cheese, bryndza, tvorog; is used in chemical, paper, the textile industry; in medicine, agriculture; as de-icing agent for municipal purposes

Table 1. Cont.

Substance	Code	Class of Additives	Commonest Uses
Sodium nitrite (NaNO ₂)	E250	preservatives	In the food industry, it is used as a colour stabilizer and preservative in meat and fish, and also antibacterial agent, preventing the growth of <i>Clostridium botulinum</i> ; is used as anti-freeze in concretes in production of construction materials and structures, as inhibitor of atmospheric corrosion; in organic synthesis and other purposes in chemical, metallurgical, medical, pulp and paper industries and other spheres
Potassium nitrite (KNO ₂)	E249	preservatives	Prevents formation of botulinum toxin (forms during vital activity of <i>Clostridium botulinum</i>); added to meat, it forms nitrosomyoglobin, which has distinct red colour (salami, sausages, etc.); for diazotization in production of azo dyes
Sodium nitrate (NaNO ₃)	E251	preservatives	As plant fertilizer; in the food, glass, metal-processing industries; for production of explosives, rocket fuel and pyrotechnic mixtures (to provide fire yellow colour)
Potassium nitrate (KNO ₃)	E252	preservatives	in the food industry, in cheese making, to slow down “blowing”; into meat and fish; in glass production; one of the components of rocket fuel; as a component of pyrotechnic mixtures and powder; as mineral fertilizer, and also to increase frost resistance of plants; in toothpastes for sensitive teeth
Ammonium bicarbonate (NH ₄ HCO ₃)	E503	stabilizers, emulsifiers	As leavening agent in the food industry; for accelerating the fermentation in wine-making; for chrome tanning in the leather industry; in pharmaceuticals, for preparing syrups, vitamins, ammonia spirit and other drugs; in cosmetics; for soil fertilization in agriculture, sometimes is included in fodders; promotes increase in rates of humus formation in soil; in the rubber technology industry, in ceramics production; for dyeing fabrics; in production of fire extinguishing agents
Sodium bisulfite (NaHSO ₃)	E228	preservatives	in the food industry, as a preservative, bleacher, antioxidant, colour stabilizer; as fertilizer in agriculture, especially for chlorophytic plants in open plots and in premises; is used to produce alum and other chemical compounds of potassium, and also as component of mixtures for making glass
Sodium bisulfate (NaHSO ₄)	E514	stabilizers, emulsifiers	In the food industry, it is added as a preservative or antioxidant; in most exported wines, it is used to prevent oxidation and to preserve taste; as reagent for reduction of heavily soluble oxides into soluble sulphates; as powder or granules as reagent that decreases pH level in swimming pools
Sodium sulfate (Na ₂ SO ₄)	E514	stabilizers, emulsifiers	As antimicrobial agent and antioxidant in the food industry; also is used to lower pH, in metal processing, in detergents and to lower water pH for effective chlorination in swimming pools and hydromassage baths
Potassium sulfate (K ₂ SO ₄)	E515	stabilizers, emulsifiers	In the EU, it is allowed as food additive; in agriculture, it is a valuable chlorine-free fertilizer; in production of glass, various alums and other potassium compounds; as flux in metalurgy
Calcium sulfate (CaSO ₄)	E516	stabilizers, emulsifiers	In the food industry, it is mostly used in bread production, and also for enriching wheat flour, cookies, cakes and bread; production of construction materials, concrete; as soil fertilizer and supports pH balance
Sodium thiosulfate (Na ₂ S ₂ O ₃)	E539	stabilizers, emulsifiers	in food production; exerts anti-toxic, anti-inflammatory and desensitization effects; is a donor of sulfate ions; is used as substrate for synthesis of non-toxic thio-compounds; antidote to poisoning by prussic acid and cyanides, arsenic, lead, mercury, iodine and bromine compounds

Table 1. Cont.

Substance	Code	Class of Additives	Commonest Uses
Sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$)	E223	preservatives	As preservative, antioxidant, bleacher and leavening agent in food production, in production of fruit juices, sweets, wine and beer; to remove tree stumps; in the fishing industry; in processing of wastewater
Potassium metabisulfite ($\text{K}_2\text{S}_2\text{O}_5$)	E224	preservatives	In the food industry; widely used in the beer brewery as stabilizer, stopping yeasting processes; to process dried fruits, and also some kinds of confectionary
Copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)	E519	stabilizers, emulsifiers	In the food industry; as colour fixator and preservative; has disinfecting, antiseptic, binding properties; is used as antiseptic in medicine and anti-bacterial Agent in horticulture; fungicide; copper-sulfate agricultural fertilizer
Tetrasodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$)	E450	stabilizers, emulsifiers	In the food industry, especially meat processing, improves organoleptic parameters, product consistency, stabilizes its colour; is used as emulsifier in food; as preservative of various meat products; for preparing processed cheese; is present in some dairy products
Sodium triphosphate ($\text{Na}_5\text{P}_3\text{O}_{10}$)	E451	stabilizers, emulsifiers	Is used as colour fixator, pH regulator, emulsifier, stabilizer in food production
Sodium borate decahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$)	E285	preservatives	In the food industry, is added to black caviar and some canned fish; to soften water; is necessary for production of glue, porcelain; as fertilizer in conditions of boron deficiency in agriculture; necessary component for cleaning agents and detergents; in substances to clean and whiten teeth; necessary component for welding steel and cast iron, and also soldering gold and silver
Talc ($\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$)	E553	stabilizers, emulsifiers	As food additive, mainly in confectionary and for gloss finish, provides gloss to caramel, is included in dragée, caramel, is allowed in production of cheese and cheese products, spices, dry milk and cream, salt and salt substitute; baby powder; to minimize friction of surfaces, prevents dust mould and sticking of rubber products during storage; filler in paper, medical, rubber, paint and lacquer industries, perfumery, cosmetics and other spheres

Data are generalized based on Brannen et al. [32], with additions of information from other sources.

The pH of manure of goats is close to neutral (Table 2). According to various authors, concentrations of phosphorus, calcium and potassium vary greatly depending on place of sample collection. While being decomposed by microorganisms with access to oxygen and in anaerobic conditions, organic substances in manure interact with inorganic substances, and therefore the concentration of acids or their salts changes over time in the same sample depending on moisture of the substrate, its temperature, dominating microorganisms and many other factors [33–36].

The larvae were cultivated in a thermostat for 10 days in 18–22 °C temperature. Larvae of *S. papillosus* (first–third ages— L_1 , L_2 , L_3), *H. contortus* (third stage L_3) and *M. capillaris* (first stage of development L_1) were isolated from feces of goats, which had been naturally infected during grazing, using Baermann's technique (Baermann test) [37]. Species of parasites were identified based on morphological peculiarities of larvae of the indicated stages [38,39]. At the same time, we took into account body size, structure of the esophagus, and also the intestine. Then, the larvae were placed in 10 mL test tubes and centrifuged at 1500 rpm for 4 min. The sediment with larvae was stirred and evenly distributed (an average *S. papillosus* of 14–35 larvae, *H. contortus* of 5–11 larvae, *M. capillaris* of 15–25 larvae) into 1.5 mL centrifugation tubes, 0.1 mL into each, to which then the tested substances were added in five repetitions for each variant of the experiment. In the experiment, we used

three concentrations of inorganic food additives: 1%, 0.1% and 0.01%. The exposure lasted 24 h, the temperature was 22 °C;. Then, we counted live and dead (immobile nematodes that had decomposition of the intestine tissue) larvae.

Table 2. Chemical composition of manure of goats.

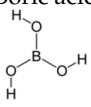

Chemical Parameter	Parameter Value	The Source of Information
pH	6.82	[33]
P; (%)	0.42–0.43	[34]
	0.36 ± 0.029	[35]
	0.12	[33]
	1.86 ± 0.01	[36]
K (%)	0.93	[34]
	0.77 ± 0.042	[35]
	0.95	[33]
	1.64 ± 0.01	[36]
Ca (%)	0.90	[33]
	3.52 ± 0.01	[36]
Na (%)	1.28	[33]
Mg (%)	1.29 ± 0.01	[36]
	0.34	[33]
O (%)	41.16 ± 0.06	[36]
C (%)	40.09 ± 0.04	[36]
H (%)	5.85 ± 0.05	[36]
N (%)	2.78–2.81	[34]
	1.95 ± 0.04	[36]
	1.94	[33]
	2.57 ± 0.182	[35]
Si (%)	1.15 ± 0.02	[36]
Zn (%)	0.06 ± 0.002	[36]
Mn (%)	0.06 ± 0.001	[36]
Fe (%)	0.18 ± 0.004	[36]
Al (%)	0.18 ± 0.004	[36]

The results were statistically analyzed using a set of Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA). The tables indicate mean value (x) ± 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

Table 3 demonstrates the results of the study of influence of alkali sodium hydroxide and potassium hydroxide. After 24 h exposure to 1% solutions of those substances, 100% of larvae of all studied species of nematodes died. However, 0.1% concentration of sodium hydroxide caused 100% death of only non-invasive larvae of *S. papillosus*. Over 72% of larvae of other nematode species, and also invasive larvae of *S. papillosus* survived the 24 h exposures to this concentration of sodium hydroxide. Lower effects against non-invasive larvae of *S. papillosus* were exhibited by potassium hydroxide. Moreover, the nematodes were affected by phosphoric acid: 1% solution of this acid decimated 80% of nematode larvae (except invasive larvae of *H. contortus*, and also *S. papillosus*, mortality of which did not exceed 5% and 27%, respectively). We observed no casualties among nematode larvae exposed to boric acid in any of the concentrations. At the same time, the lowest susceptibility to alkalis and acids were seen among non-invasive larvae of *S. papillosus*.

Table 3. Mortality of larvae of *S. papillosus*, *H. contortus*, *M. capillaris* (%) during 24 h laboratory experiment under the influence of alkalis and 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 hydroxide NaOH	L ₁₋₂ of <i>S. papillosus</i>	30.2 ± 11.3 ^a	100.0 ± 0.0 ^b	100.0 ± 0.0 ^b	56.0 ± 8.0 ^c	0.1%
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	100.0 ± 0.0 ^b	28.6 ± 15.1 ^c	0.0 ± 0.0 ^a	1%
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	100.0 ± 0.0 ^b	2.1 ± 5.1 ^a	0.0 ± 0.0 ^a	1%
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	100.0 ± 0.0 ^b	25.5 ± 7.8 ^c	1.3 ± 1.8 ^a	1%
Potassium hydroxide KOH	L ₁₋₂ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	100.0 ± 0.0 ^b	23.3 ± 4.0 ^c	0.0 ± 0.0 ^a	1%
	L ₃ of <i>S. papillosus</i>	16.7 ± 3.2 ^a	100.0 ± 0.0 ^b	16.0 ± 7.5 ^a	15.6 ± 4.8 ^a	1%
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	100.0 ± 0.0 ^b	10.9 ± 4.8 ^c	0.0 ± 0.0 ^a	1%
	L ₁ of <i>M. capillaris</i>	7.5 ± 11.2 ^a	100.0 ± 0.0 ^b	29.4 ± 5.2 ^a	9.2 ± 10.3 ^a	1%
Boric acid 	L ₁₋₂ 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>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	—
Phosphoric acid 	L ₁₋₂ of <i>S. papillosus</i>	5.7 ± 3.6 ^a	87.5 ± 8.4 ^b	23.3 ± 6.2 ^c	9.8 ± 1.1 ^a	1%
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	26.7 ± 25.3 ^{ab}	4.0 ± 8.9 ^a	9.0 ± 12.4 ^a	—
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	4.2 ± 10.2 ^a	2.1 ± 5.1 ^a	0.0 ± 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	100.0 ± 0.0 ^b	6.8 ± 5.3 ^c	0.0 ± 0.0 ^a	1%

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

Similar results were obtained for the use of sodium metabisulfite: 1% solution caused death to over 85% of larvae of *S. papillosus*, *M. capillaris* and also over 69% of *H. contortus*. This inorganic food additive in 0.1% concentration also produced death of 62% of non-invasive larvae of *S. papillosus*. The rest of the salts of inorganic food additives were less effective compared with alkalis. Among those compounds, sodium sulfate had the strongest effects on *S. papillosus* and *M. capillaris*. This substance in 1% solution killed 100% of *S. papillosus* larvae of different stages and over 66% of first-age *M. capillaris* larvae. At the same time, 0.1% solution of sodium sulfate had a lethal effect on 73% of non-invasive larvae (first-second ages) of *S. papillosus* (Table 4).

Table 4. Mortality of larvae of *S. papillosus*, *H. contortus* and *M. capillaris* (%) during 24 h laboratory experiment under the influence of salts, 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%)
Potassium chloride KCl	L ₁₋₂ of <i>S. papillosus</i>	15.3 ± 5.6 ^a	18.6 ± 7.9 ^a	17.4 ± 5.8 ^a	17.4 ± 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>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	—
Calcium chloride CaCl ₂	L ₁₋₂ of <i>S. papillosus</i>	18.9 ± 5.7 ^a	54.9 ± 3.7 ^b	16.6 ± 3.0 ^a	15.9 ± 3.0 ^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	—

Table 4. Cont.

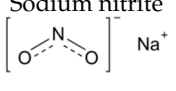
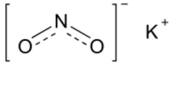
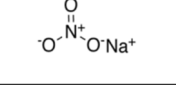
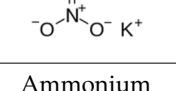
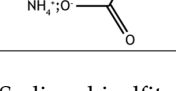
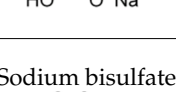
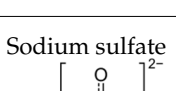
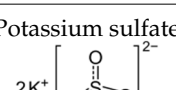
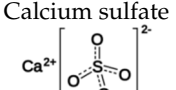
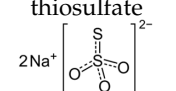

Substance	Nematode Species	Mortality of Nematode Larvae in Control, %	Mortality of Nematode Larvae in 1% Solution, %	Mortality of Nematode Larvae in 0.1% Solution, %	Mortality of Nematode Larvae in 0.01% Solution, %	Lowest Effective Concentration (Mortality Over 70%)
Sodium nitrite 	L _{1–2} of <i>S. papillosus</i>	10.7 ± 5.5 ^a	57.3 ± 15.9 ^b	44.1 ± 20.0 ^b	19.2 ± 11.8 ^{ab}	–
	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 nitrite 	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	37.4 ± 2.3 ^b	19.9 ± 2.6 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	10.7 ± 1.8 ^b	1.8 ± 2.1 ^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	50.0 ± 0.0 ^b	29.5 ± 11.8 ^c	8.4 ± 7.9 ^{ac}	–
Sodium nitrate 	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	35.6 ± 2.2 ^b	26.2 ± 1.3 ^c	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	9.9 ± 1.8 ^b	5.3 ± 1.8 ^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>	6.7 ± 9.1 ^a	32.6 ± 10.4 ^b	7.9 ± 11.4 ^{ab}	6.2 ± 8.5 ^a	–
Potassium nitrate 	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	12.9 ± 1.6 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^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	–
Ammonium bicarbonate 	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	92.1 ± 2.3 ^b	19.4 ± 6.6 ^c	4.8 ± 2.0 ^d	1%
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	36.5 ± 5.6 ^b	15.5 ± 17.6 ^{ab}	0.0 ± 0.0 ^a	–
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	5.2 ± 8.5 ^a	2.8 ± 6.8 ^a	0.0 ± 0.0 ^a	–
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	16.0 ± 9.1 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
Sodium bisulfite 	L _{1–2} of <i>S. papillosus</i>	0.0 ± 0.0 ^a	19.7 ± 1.4 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	–
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	8.5 ± 2.4 ^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	94.2 ± 6.2 ^b	8.8 ± 7.2 ^c	9.5 ± 9.7 ^{ac}	1%
Sodium bisulfate 	L _{1–2} of <i>S. papillosus</i>	18.9 ± 5.7 ^a	20.9 ± 5.7 ^a	20.2 ± 5.5 ^a	17.0 ± 3.7 ^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	–
Sodium sulfate 	L _{1–2} of <i>S. papillosus</i>	19.6 ± 1.4 ^a	100.0 ± 0.0 ^b	73.6 ± 5.1 ^c	19.5 ± 5.4 ^a	0.1%
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	100.0 ± 0.0 ^b	12.0 ± 2.9 ^c	0.0 ± 0.0 ^a	1%
	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>	15.9 ± 10.5 ^a	66.3 ± 9.0 ^b	19.5 ± 4.2 ^a	18.0 ± 7.7 ^a	–
Potassium sulfate 	L _{1–2} of <i>S. papillosus</i>	34.0 ± 6.5 ^a	49.3 ± 5.5 ^{ab}	38.8 ± 4.1 ^a	38.7 ± 6.8 ^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	–
Calcium sulfate 	L _{1–2} of <i>S. papillosus</i>	34.0 ± 6.5 ^a	60.6 ± 7.9 ^b	43.0 ± 8.0 ^{ab}	39.6 ± 4.0 ^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	–
Sodium thiosulfate 	L _{1–2} of <i>S. papillosus</i>	11.6 ± 7.0 ^a	12.8 ± 5.3 ^a	15.3 ± 7.9 ^a	14.4 ± 9.8 ^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	27.7 ± 3.4 ^a	26.3 ± 7.1 ^a	21.4 ± 4.6 ^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 metabisulfite $2\text{Na}^+ \left[\text{O}=\text{S}(\text{O})_2\text{O} \right]^{2-}$	L ₁₋₂ of <i>S. papillosus</i>	24.5 ± 4.1 ^a	100.0 ± 0.0 ^b	62.1 ± 10.5 ^c	28.4 ± 8.3 ^a	1%
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	85.8 ± 8.2 ^b	21.7 ± 12.9 ^c	0.0 ± 0.0 ^a	1%
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	69.8 ± 34.3 ^b	5.8 ± 8.1 ^a	0.0 ± 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	100.0 ± 0.0 ^b	11.0 ± 4.2 ^c	0.0 ± 0.0 ^a	1%
Potassium metabisulfite $2\text{K}^+ \left[\text{O}=\text{S}(\text{O})_2\text{O} \right]^{2-}$	L ₁₋₂ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	87.6 ± 1.7 ^b	15.6 ± 0.8 ^c	0.0 ± 0.0 ^a	1%
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	12.8 ± 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	5.4 ± 7.4 ^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 ± 16.6 ^b	10.1 ± 6.2 ^c	8.6 ± 12.1 ^{ac}	—
Copper (II) sulfate pentahydrate $\text{H}-\text{O}-\text{P}(\text{O})(\text{OH})_2$	L ₁₋₂ of <i>S. papillosus</i>	6.6 ± 1.3 ^a	100.0 ± 0.0 ^b	7.0 ± 0.7 ^a	0.0 ± 0.0 ^c	1%
	L ₃ of <i>S. papillosus</i>	6.9 ± 6.6 ^a	100.0 ± 0.0 ^b	14.4 ± 4.8 ^a	0.0 ± 0.0 ^c	1%
	L ₃ of <i>H. contortus</i>	0.0 ± 0.0 ^a	10.0 ± 13.2 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	—
	L ₁ of <i>M. capillaris</i>	0.0 ± 0.0 ^a	22.1 ± 24.7 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	—
Tetrasodium pyrophosphate $\left[\text{O}-\text{P}(\text{O})_2\text{O}-\text{P}(\text{O})_2\text{O} \right]^{4-} \left[\text{Na}^+ \right]_4$	L ₁₋₂ of <i>S. papillosus</i>	18.9 ± 5.7 ^a	80.2 ± 6.6 ^b	46.9 ± 7.2 ^c	16.8 ± 6.0 ^a	1%
	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	6.6 ± 5.4 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	—
Sodium triphosphate $\left[\text{O}-\text{P}(\text{O})_2\text{O}-\text{P}(\text{O})_2\text{O}-\text{P}(\text{O})_2\text{O} \right]^{5-} \left[\text{Na}^+ \right]_5$	L ₁₋₂ of <i>S. papillosus</i>	34.0 ± 6.5 ^a	75.6 ± 2.9 ^b	28.0 ± 5.7 ^a	34.5 ± 4.1 ^a	1%
	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	—
Sodium borate decahydrate $\left[\text{Na}(\text{H}_2\text{O})_4 \right]^{+} \left[\text{B}_3\text{O}_6 \right]^{2-}$	L ₁₋₂ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	3.9 ± 3.1 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	—
	L ₃ of <i>S. papillosus</i>	0.0 ± 0.0 ^a	31.4 ± 2.6 ^b	15.0 ± 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	19.0 ± 20.7 ^a	15.4 ± 14.6 ^a	13.7 ± 14.0 ^a	—
Talc $\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$	L ₁₋₂ of <i>S. papillosus</i>	14.2 ± 5.9 ^a	17.4 ± 10.9 ^a	12.8 ± 5.1 ^a	17.8 ± 6.8 ^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	20.4 ± 4.7 ^a	15.1 ± 5.3 ^a	14.7 ± 7.2 ^a	—

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

Over 24 h, copper sulfate pentahydrate in 1% concentration killed all stages of *S. papillosus*. Ammonium bicarbonate, calcium chloride, sodium nitrite, calcium sulfate, potassium metabisulfite, tetrasodium pyrophosphate, sodium triphosphate in 1% solutions caused death to only non-invasive larvae of *S. papillosus*. Lower concentrations of those additives had weak effects on survivability of larvae of the studied species of nematodes. Sodium bisulfite and potassium nitrite were effective only against *M. capillaris*. Their 1% solutions caused death to over 94% of the larvae (Table 4).

4. Discussion

The fight against pathogens of infectious and parasitic diseases that inflict great economic losses on agricultural and livestock farms is underway all around the globe [20,40]. At the same time, agriculture is losing large amounts of meat and dairy products [25,41,42]. There are numerous publications on the use of chemically synthesized substances, nematocides and anthelmintic drugs, which have been accumulating in soil, water bodies and also tissues of living organisms [43,44]. During the migration through food chains, those sub-

stances end up in the organisms of animals and contaminate agricultural products [29,30]. Over recent years, alternative substances to combat pests have become broadly distributed: there are compounds that already exist in nature, such as medicinal plants [45–47], their essential oils [48,49], and also substances used in the food industry [50,51].

Some inorganic food additives, including alkalis, acids and salts may have negative effects on nematode larvae—parasites of agricultural animals. McSorley and McGovern [52] studied the effects of ammonium bicarbonate on nematode parasitizing *Cucurbita pepo* L. and *Catharanthus roseus* (L.) G. Don. According to the results of their studies, use of ammonium bicarbonate led to lower number of phytoparasitic nematodes of *Belonolaimus longicaudatus* Rau, 1958. In our experiments, ammonium bicarbonate also caused a significant impact on larvae of *S. papillosus*. Nonetheless, larvae of other species of nematodes (*H. contortus*, *M. capillaris*) were more resistant to this substance. This food additive, and also a number of other compounds used in the food industry (sodium nitrite and nitrate) have demonstrated effective fungicidal effects against *Fusarium oxysporum* f. sp. *cucumbrum*, *F. oxysporum* f. sp. *niveum* and *F. oxysporum* f. sp. *melonis* [53]. Aslam et al. [54] and Sun et al. [55] also used ammonium bicarbonate against the rot pathogen of carrot (*Daucus carota* L.), *Pectobacterium carotovorum* (Jones, 1901) Waldee, 1945. In those studies, two substances recommended as safe (GRAS) were used: sodium bicarbonate and ammonium bicarbonate. The experiments were carried out on carrots that had been inoculated and infected naturally. Carrots that had not been treated with the studied substances became infected nine days later. Carrots that had been inoculated prior to the treatment with 2% ammonium bicarbonate were observed to have 50% decrease in morbidity after nine days of storage. At the same time, during the storage of carrots, organoleptic parameters and physical–chemical properties of the treated tubers did not change.

Ignatowicz and Pankiewicz-Nowicka [56] studied the effects of inorganic salts on biology and development of Acari, particularly the influence of calcium chloride on fertility and the development of eggs of *Acarus siro* Linnaeus, 1758. Addition of this substance in 1.5–6.0% amounts to the diet of the Acari led to over 50% decrease in their fertility. The life span of *A. siro* decreased as well. As alternative compounds against mites *Polyphagotarsonemus latus* (Banks, 1904) and aphids *Myzus persicae* (Sulzer, 1776), salts and inorganic acids were used, including boric and ascorbic acids, and also potassium sorbate. Those same substances used in the food industry were studied in field experiments against Acari and aphids infesting potatoes. Populations of these pests were observed to decrease [57,58].

Boric acid is used in spheres other than the food industry. This acid is quite often used against helminths of cattle which parasitize animals' eyes. According to Karmaliyev et al. [59], solution of boric acid is used in order to wash *Thelazia* sp. off of the conjunctival sac. To remove helminths of this genus, Singh and Khindria [60] also used 2–3% solution of boric acid. However, boric acid was ineffective at combatting nematode larvae—parasites of the gastrointestinal tract and respiratory system of ruminants—in the environment. Twenty-four-hour exposure of its 1% solution led to death of larvae of all researched species of helminths. Orthophosphate acid is also recorded not only as a food additive, but also as an insecticide [61]. We also observed its action against non-invasive larvae of *S. papillosus*. However, no effects were observed against other stages of *S. papillosus*, and also larvae of *H. contortus* and *M. capillaries*.

Substances allowed in organic arable farming and processing include many organic food additives. In most cases, use of inorganic chemically synthesized compounds that may be a source of contamination of organic products or environmental objects is prohibited. Potassium metabisulfite belongs to inorganic food additives allowed by the IFOAM Standards Committee (International Federation of Organic Agriculture Movements) to be used in organic arable farming [62]. In our experiment, this substance had the greatest impact on non-invasive stages of *S. papillosus*: over 80% of first–second stage larvae died when exposed to its 1% solution. Therefore, potassium metabisulfite may be further studied for purposes of combating nematode larvae at early stages of development in the environment of organic farms.

Thus, food additives introduced into the environment together with unused food products of humans or as a result of utilization in various spheres of human activity (in agricultural farms, construction, chemical industries, etc.) cause changes in life of soil stages of the development of parasitic nematode larvae, and also other invertebrates [63]. High concentrations of the substances (1%) we studied are likely to be found in soil only locally, near the places of non-sorted solid municipal wastes. However, some studied substances increase the mortality of nematode larvae even in 0.1% concentrations, indicating significant risk from their use to maintenance of the normal quantity of soil nematodes—normal component of communities of soil invertebrates.

Regarding the hypotheses we formulated in the Introduction, we should emphasize the following. A high occurrence of inorganic substance in the wild did not turn out to be a guarantee of its safety for larvae of parasitic nematodes: for example, 1% concentration of phosphorus acid killed L₁₋₂ of *S. papillosus* and L₁ of *M. capillaris* (Table 3); 1% concentration of ammonium bicarbonate caused death to L₁₋₂ of *S. papillosus*; and 0.1% and 1% concentrations of sodium sulfate were lethal to L₁₋₂ and L₃ of *S. papillosus*.

On the other hand, the presumption that substances that do not occur in natural soils could cause death to soil larvae of parasitic nematodes was not found to be correct entirely and in general was not confirmed for such compounds as boric acids (Table 3), sodium thiosulphate (Table 4), which did not increase mortality of any of the three species of nematodes we studied. However, sodium metabisulfite—which does not occur in natural soil—was efficient against L₁₋₂ and L₃ of *S. papillosus* in 1% concentration, as well as L₁ of *M. capillaris*; potassium metabisulfite produced death of L₁₋₂ of *S. papillosus* in 1% concentrations; copper (II) sulfate pentahydrate, which is not found in natural soils, exerted a lethal effect for L₁₋₂ and L₃ of *S. papillosus* in 100% of the cases, but caused no significant increase in mortality of L₁ of *M. capillaris* and L₃ of *H. contortus*; sodium borate decahydrate in natural soils does not occur as well, but killed no nematode larvae. Substances that do not occur in natural soils and are highly soluble in water can cause death of nematodes or have no effect on their vitality at all.

As with our third hypothesis that pH would poorly affect the survivability of the nematodes, we turned out to be partly right. Alkaline reaction of the environment increased mortality of all the studied species of nematodes in one-percent concentration (for sodium hydroxide and potassium hydroxide in Table 3), whereas acids had a lower effect on survivability of the species of nematodes we studied.

5. Conclusions

Inorganic food additives such as alkalis, acids and salts have various effects on the gastrointestinal tract and respiratory system of ruminants. The strongest effects on survivability of larvae in in vitro conditions were caused by sodium hydroxide, potassium hydroxide and also sodium metabisulfite. Twenty-four-hour exposure to their 1% solutions killed over 69% of larvae of *S. papillosus*, *H. contortus* and *M. capillaris* of various stages of the development. Sodium sulfate was effective against larvae of various ages of *S. papillosus*, and also first-stage larvae of *M. capillaris*. Copper sulfate pentahydrate in 1% concentration caused the death of all development stages of *S. papillosus*. Thus, the results we obtained revealing the strong effect of alkalis on nematode larvae could be of not only scientific but also practical significance for farms in combating parasites of ruminants. The data we obtained are relevant for future ecologic studies of the effects alkalis have on other soil nematodes, structural elements of natural biocenoses.

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