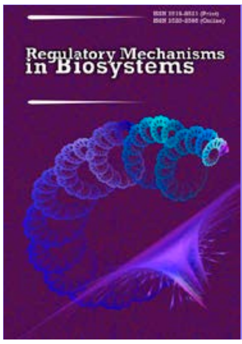

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst., 9(1), 118–123
doi: 10.15421/021817

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

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Article info

Received 03.01.2018
Received in revised form 29.01.2018
Accepted 31.01.2018

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Boyko, O. O., & Brygadyrenko, V. V. (2018). The impact of certain flavourings and preservatives on the survivability of larvae of nematodes of Ruminantia. Regulatory Mechanisms in Biosystems, 9(1), 118–123. doi: 10.15421/021817

Annualy, helminthic diseases are one of the causes of economic losses in agriculture. Ruminantia are most often observed to be infected with nematodes of the gastrointestinal tract, including *Strongyloides papillosus* and the representatives of the Strongylida order. Identifying factors which could cause a decrease in the level of infection of agricultural animals with helminthiases would allow stock-raising facilities to regularly achieve high quality dairy and meat production in sufficient quantity. On the basis of this study, we determined the impact of flavourings and source materials approved for use in and on foods (isoamyl alcohol, isoamyl acetate, raspberry ketone, trilon B, methylparaben) on the survivability of larvae of *Strongyloides papillosus* and *Haemonchus contortus*, parasitic nematodes of Ruminantia animals. Among these substances, the lowest efficiency against the nematode larvae was found in isoamyl alcohol, isoamyl acetate and trilon B. A stronger effect was caused by methylparaben (for L_3 *S. papillosus* $LD_{50} = 0.67 \pm 0.04\%$, L_1 and L_2 *S. papillosus* – $LD_{50} = 0.0038 \pm 0.0008\%$, L_3 *H. contortus* – $LD_{50} = 0.89 \pm 0.15\%$). Minimum efficient dosage of the solutions was 10 g/l. Significant antihelminthic properties were manifested by raspberry ketone (for L_3 *S. papillosus* $LD_{50} = 1.00 \pm 0.72\%$, L_1 and L_2 *S. papillosus* – $LD_{50} = 0.07 \pm 0.06\%$, L_3 *H. contortus* – $LD_{50} = 0.39 \pm 0.26\%$). The results show that there is considerable potential for further studies on the antiparasitic properties of these substances against nematodes in the conditions of farming enterprises and agricultural complexes.

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

Introduction

Throughout the world a daily struggle takes place against parasitic diseases of agricultural animals. These infestations cause significant economic damage to farming enterprises and large stock-raising facilities, which consequently fall short of their potential in meat and dairy production. The most common parasitic diseases are helminthiases (Vercruysse et al., 2001; Biffa et al., 2007; Van der Voorta et al., 2013; Boyko et al., 2016). Ruminantia in Ukraine and other countries of Europe are regularly observed to be infected with the following nematodes of gastrointestinal tract: Haemonchosis, Nematodirus, Trichostrongyloidosis, Chabertiosis, Bunostomosis, Protostrongylidoses, Strongyloides, etc. (Lindqvist et al., 2001; Bhutto et al., 2002). Some of the abovementioned parasites are related to the feeding of nematodes on the blood of mammal hosts. As a result, the biochemical indicators of the milk of cattle, sheep and goats change (Boyko et al., 2016). The quality of the meat products decreases as well. The helminths constantly release a significant number of toxins into the host's organism, causing intoxication (Faye et al., 2003; Cringoli et al., 2008).

In the struggle against helminthic diseases, therapeutic and preventive measures play a significant role. They are aimed at chemotherapy and chemoprophylaxis with the use of anthelminthic preparations. The most common (Ploeger et al., 1990; Kloosterman et al., 1996; Veneziano et al., 2004) preparations against helminths are broad-spectrum preparations on the basis of macrocyclic lactones (Ivermectin, Doramectin, Abamectin, etc), Benzimidazoles (Albendazole, Mebendazole, Fenbendazole). Modern veterinary medicine also applies alternative methods against helminths using herbaceous preparations. However, the extent of their impact is differently interpreted by various authors (Rahmann & Seip, 2006; Burke et al., 2009; Cheng et al., 2009; Lu et al., 2010; Chhetri et al., 2015; González-Coloma et al., 2017). For preventing helminthic infestations, it is recommended to cultivate the pastures, plough the land on their territories, provide a pen-system of grazing, conduct mechanical cleaning from bushes, rocks, drying, and taking measures against intermediate hosts and other methods. Our previous studies devoted to the impact of food additives on the survivability of the larvae of the nematode of pigs *Strongyloides ransomi* (Schwartz and Alicata, 1930), and eggs of *Ascaris suuum* Goeze, 1782 indicated positive results for certain flavourings, preservatives and other types of food additives used in the food industry. Therefore, a relevant issue today is determining their impact on other species of nematodes often found in agricultural animals (Boyko et al., 2017).

Materials and methods

In the summer of 2017, we collected faeces of Ruminantia on the territory of Dnipropetrovsk oblast of Ukraine to the amount of 100 g from every individual (n = 56). The material was transported in plastic containers at a temperature of 22–24 °C to the parasitelogical laboratory of Dnipro State Agrarian-Economic University. The samples with helminths for use in the experiment were identified using the McMaster method. For the study, we selected third age larvae (L_3) of *Haemonchus contortus* (Rudolphi, 1803) from the Strongylida order and larvae of first, second and third age (L_1, L_2, L_3) of *Strongyloides papillosus* (Wedl, 1856) from the Rhabditida order (Van Wyk & Mayhew, 2013) (Fig. 1). For the experiment, the larvae were cultivated during 8 days at a temperature of 22–24 °C. The larvae material was collected using the Baermann test (Zajac & Conboy, 2011).

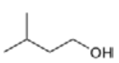
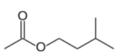
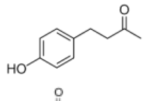
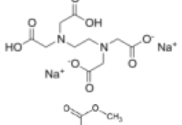
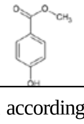
a

b

Fig. 1. *Strongyloides papillosus* (Wedl, 1856) of different ages (*a*) and *Haemonchus contortus* (Rudolphi, 1803) L_3 (*b*): bar – 10 µm

Table 1

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

Substance name	Chemical formula	Structural formula	Properties	Content	Usage	
					in food industry	in medicine
Isoamyl alcohol (3-methylbu-tan-1-ol)	$C_5H_{12}O$		optically inactive colourless substance with unpleasant odour	fusel oils	used for preparing extractions with pleasant fruit odour	no data on usage
Isoamyl acetate (3-methyl-butyl acetate)	$C_7H_{14}O_2$		colourless substance with sharp pear odour	in some fruits	pear extraction for produ-cing fruit water, caramel, etc.	no data on usage
Raspberry ketone (4-(4-hydroxyphenyl) butan-2-one)	$C_{10}H_{12}O_2$		colourless substance with citrus odour	in red raspberries	as a food additive with fruit flavour	used in cosmetology
Trilon B (2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetra-acetic acid), E_{385}	$C_{10}H_{16}N_2Na_2O_8$		white crystalline powder or crystals of white colour	–	in food preservation, as antioxidant	in the production of medical preparations and in cases of heavy metal intoxication, in dentistry, as a preservative in eye preparations
Methylparaben, E_{218}	$C_8H_8O_3$		white crystalline substance with distinctive odour	in the roots of *Oxalis tuberose*	as a preservative	as an antiseptic

Note: * – properties of the substances are given according to Lide, 1980; Fahlbusch et al., 2002; Soni et al., 2002; Catalog of Organics and Fine Chemicals, 2004; Nomenclature of Organic Chemistry, 2014.

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

The statistical analysis of the results was performed through a set of Statistica 8.0 (StatSoft Inc., USA), the figures is show the median, 25% and 75% quartiles, minimum and maximum values. LD_{50} (%) was calculated as average (x) ± standard deviation (SD).

Results

The results indicated a complete absence of anthelminthic properties in isoamyl alcohol (Fig. 2*a*) and isoamyl acetate. With exposure to isoamyl alcohol, we observed around 60% vital larvae of L_3 *S. papillosus* in 1% solution. Less resistant to the impact of isoamyl alcohol were L_1 and L_2 *S. papillosus*. The percentage of the surviving larvae of these two stages after 24 hours of exposure to 1% solution was 55%. With further solutions of isoamyl alcohol, 80% of *S. papillosus* larvae survived. Larvae of L_3 *H. contortus* were found to be the most resistant to different concentrations of this substance. At 0.0001–1% solution of this alcohol, 100% of them survived.

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

1% solution of raspberry ketone caused 100% mortality only to L_1 and L_2 *S. papillosus*. 30–50% of L_3 *S. papillosus* and *H. contortus* survived in this concentration. In 0.01% solution of this substance, over 80% of the Ruminantia nematode larvae survived. 0.0001% concentration of raspberry ketone in 100% of cases did not affect the survivability of these parasites (Fig. 2*c*).

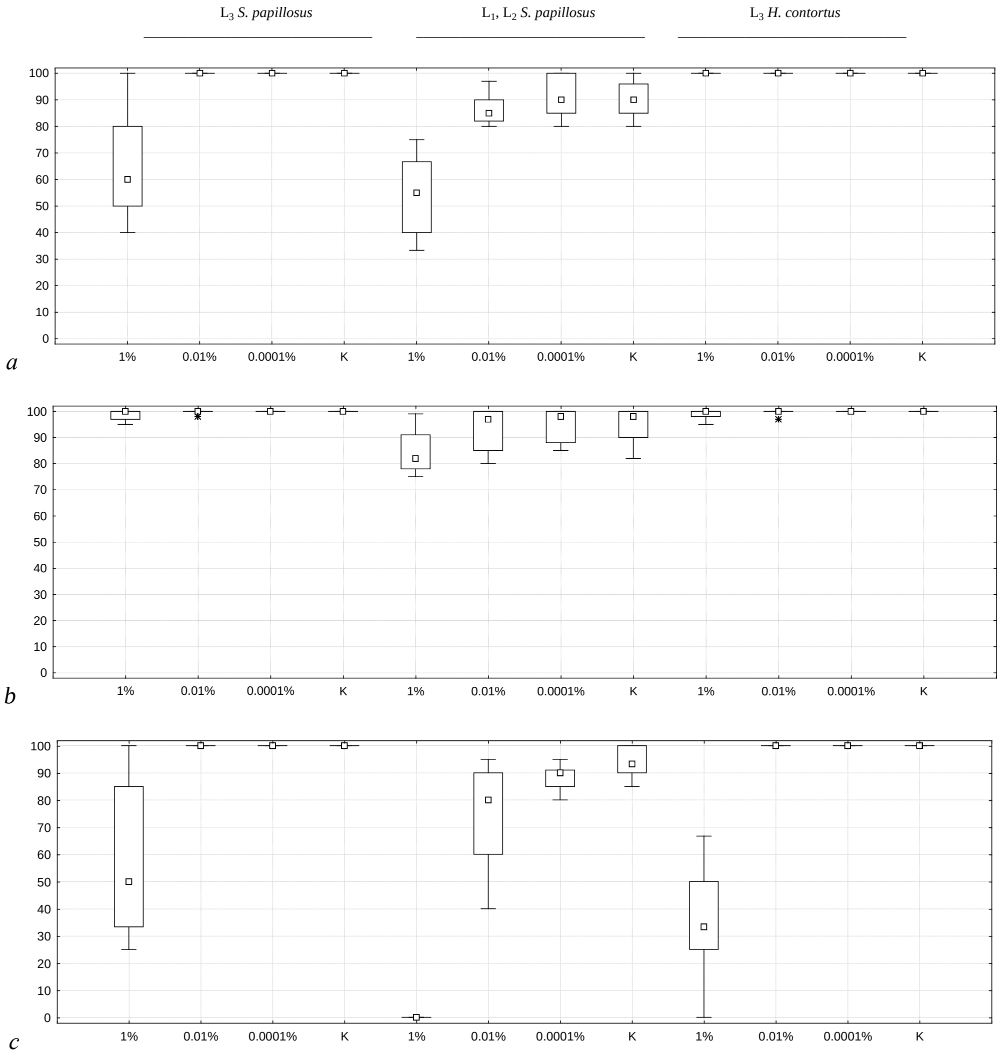


Fig. 2. The effect of flavourings on the survivability of Ruminantia nematodes: *a* – isoamyl alcohol, *b* – isoamyl acetate, *c* – raspberry ketone; the ordinate axis shows the percentage of nematode larvae which survived over the 24-hour experiment; the abscissa axis shows the concentration of the active substance in the solution (%), K – control, where the concentration of the active substance equals 0%; L_3 – invasive larvae of *S. papillosus* or *H. contortus*, L_1, L_2 – non-invasive larvae of *S. papillosus*; the small square in the center corresponds to the median, the lower and upper borders of the large rectangular correspond to the first and the third quartiles, respectively, vertical line segments, directed up and down from the rectangular, correspond to minimum and maximum values (n = 8)

The second stage of the experiment was determining the anthelminthic properties of some preservatives (trilon B and methylparaben). About 60% of L_1 and L_2 *S. papillosus* larvae died after 24 hours in 1% solution of trilon B. At the same concentration, all the rest of larvae survived in the percentage of 75–100%. The most resistant to 1% solution of trilon B were the larvae of *H. contortus*

(100% larvae survived). The next solutions of trilon B had no positive effects – all larvae survived (Fig. 3*a*). The solution of methylparaben in 1% and 0.01% concentrations caused death of non-invasive *S. papillosus* (Fig. 3*b*) larvae in 100% of cases. Invasive larvae of this species, similarly to *H. contortus* larvae are sensitive to methylparaben only at its maximum, 1% concentration of the active substance. The analysis of the study results indicated complete absence of anthelminthic properties in isoamyl alcohol and trilon B. Minimum LD_{50} (%) indicators for L_3 *S. papillosus* were registered for isoamyl acetate, raspberry ketone и methylparaben (Table 2).

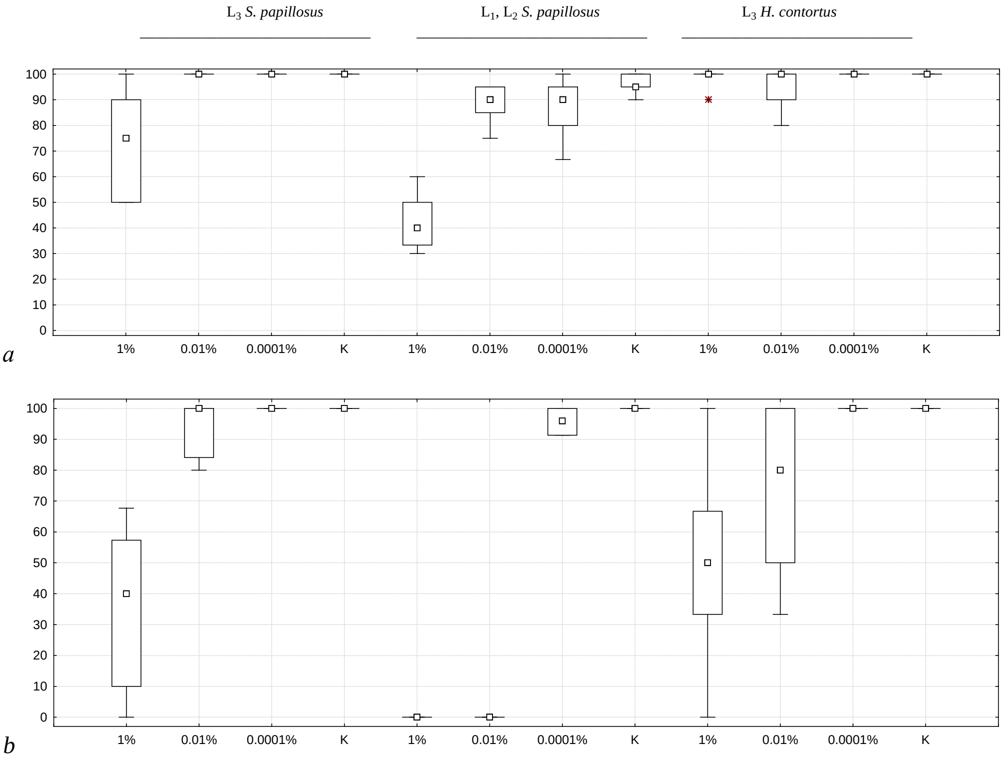


Fig. 3. The effect of trilon B (*a*) and methylparaben (*b*) on the survivability of Ruminantia nematode larvae: see notes to Fig. 1

Table 2
LD_{50} (%, x ± SD) for *S. papillosus* and *H. contortus* larvae in laboratory experiment during 24 hours

Substance	*S. papillosus*, L_3	*S. papillosus*, $L_1 + L_2$	*H. contortus*, L_3
isoamyl alcohol	–	–	–
isoamyl acetate	–	–	–
raspberry ketone	1.00 ± 0.72	0.07 ± 0.06	0.39 ± 0.26
trilon B	–	0.80 ± 0.46	–
methylparaben	0.67 ± 0.04	0.0038 ± 0.0008	0.89 ± 0.15

Discussion

Therefore, according to the results of our studies and analysis of the data from the literature, the additives used in the food industry can affect parasites, including nematode larvae of Ruminantia, in a certain concentration. Data on using food additives with the purpose of affecting the parasites are quite limited. Their impact on parasitic Acari and insects has been studied by Lee et al. (2008), Knoblauch and Fry (2011), Shen et al. (2012), Belkind et al. (2013) et al. Shen et al. (2012) indicate the significant effect of cinnamaldehyde against parasitic Acari *Psoroptes*. LD_{50} equals 107 mg/ml, (with 24 hours exposure) for Acari of this genus. Also, this food additive has been studied by Na et al. (2011) as an acaricide preparation against *Dermanyssus* of birds. LD_{50} for *Dermanyssus* sp. was 0.54 mg/ml (with 24-hour exposure). According to Lee (2004), p-anisaldehyde food additive is capable of acaricide properties. Other works are devoted to the impact of the food additive cinnamaldehyde on larvae of blood-sucking insects. LD_{50} for larvae of mosquitoes was 40.8 mg/ml. Taylor (2009) used benzyl alcohol against fleas and also indicated that this additive has insecticidal properties. Benzaldehyde has been proved to have an impact on insects. It was used against *Galleria mellonella* (Linnaeus, 1758). The authors of these studies, Ullah et al. (2015), recommended the additive for the compound of insecticidal preparations. Lee et al. (2008) have also used benzaldehyde (LD_{50} with 48-hour exposure – 0.004–0.200 mg/sm^2 against *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera, Curculionidae). Anthelminth properties of benzyl alcohol additive were proved by Chalquest (2002). Pedersen and Woldum (2011) recommend using it as solvent of preparations against parasites.

Food additives are often used as antimicrobial agents. Their impact on microorganisms has been studied by Chiang et al. (2005), Sato et al. (2006), Somolinos et al. (2008), Si et al. (2009), Belletti et al. (2010) and many other authors. Ribeiro et al. (2016) have studied antimicrobial, antifungi, and also insecticidal impact of 83 compounds from different tissues of *Ricinus communis*. Some of them are used as additives in the food industry. They include alkaloids, terpenoids, flavonoids, benzoic acid derivatives, coumarins, tocopherols, and fatty acids. The antimicrobial properties of cinnamaldehyde against *Escherichia coli* and *Salmonella enterica* were studied by Manu (2016). For obtaining antiseptic and fungicidal effect, E_{218}, a methylparaben preservative, is used (Shapiro et al., 2002; Posey et al., 2005; Kromidas et al., 2006; Rebbeck et al., 2006; Ishiwatari et al., 2007; Meyer et al., 2007; Gopalakrishnan et al., 2012). It is also used in the composition of insecticides (Bell, 1990). According to the results of our studies, this substance also affects other parasitic nematodes of Ruminantia.

The impact of ethylenediaminetetraacetic acid (EDTA or trilon B) on *Cryptococcus* has been studied by Lai et al. (2016). Currently, fungal diseases are difficult to treat, and such treatment is conducted using expensive preparations. Therefore, these authors' work was aimed at intensifying the effect of modern preparations by using them with ethylenediaminetetraacetic acid and other synergic agents for decreasing the therapeutic dose, increasing the efficiency and preventing development of *Cryptococcus* resistance. The results of our experiments indicated that usage of trilon B did not cause any death of parasitic nematodes. Our study also indicates a high level of anthelminthic impact of methylparaben and raspberry ketone. These substances affect not only microorganisms and are used as a fungicide, but are also, according to the results of our tests, capable of having an effect on *S. papillosus* and *H. contortus*, parasitic nematodes of Ruminantia.

Conclusions

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

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