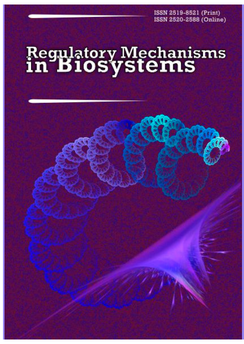

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Changes in the viability of *Strongyloides ransomi* larvae (Nematoda, Rhabditida) under the influence of synthetic flavourings

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One of the most common nematodes of pigs globally is *Strongyloides ransomi* Schwartz and Alicata 1930. It usually causes aggravation of physiological indicators of its hosts and damage to their immune system. Also it is a good modelling object for the evaluation of the antiparasitic activity of new antihelminthic drugs. We conducted laboratory experiments to assess the effect of flavouring additives with flower odour (benzaldehyde, citral, D-limonene and β-ionone) upon the viability of *S. ransomi* larvae. The mortality rate was calculated for 24 hours exposure at four concentrations of each substance (10, 1, 0.1 и 0.01 g/l) with eight replications. The lowest LD_{50} values were obtained for citral (97 mg/l) and benzaldehyde (142 mg/l). These substances are recommended for further evaluation of their antihelminthic effect in experiments using laboratory animals. Unlike other substances, the effect of β-ionone and D-limonene even at a concentration of 10 g/l after 24 hours caused the death of <50% of *S. ransomi* larvae. The study of flavouring additives with flowery odour, which are permitted to be used in food for humans and also to be used in cosmetics, is a promising field for research aimed at the development of new antiparasitic drugs.

Keywords: nematodes; antiparasitic activity; flavouring agents; benzaldehyde; citral; D-limonene; β-ionone

Introduction

Helminthiasis causes significant economic damage to animal husbandry (Faye et al., 2003; Veneziano et al., 2004; Charlier et al., 2007; Cringoli, 2008; Yamov and Antropov, 2008; Ponomar et al., 2014*a*, *b*; Boyko et al., 2016). Pig farming is one of the most common sources of meat products. Among the parasites of pigs, the most common are nematodes, including strongyloidiasis agents (Knecht et al., 2011; Eysker et al., 2011; Samsonovich, 2012*a*; Maslova et al., 2015). Parasitisation by *Strongyloides ransomi* (Schwartz and Alicata, 1930) causes loss of concentration of albuminous and albumin fractions in the blood of piglets, loss of erythrocytes, hemoglobin, albumin, phagocytic and lysozyme activity and immune reaction (Samsonovich, 2012*b*).

Therefore determining the viability of helminths is significant for controlling their population, both in the host, and in the environment. Nowadays, synthetic antiparasitic drugs (Ponomar et al., 2013) are used and experiments of identifying the antihelminthic properties of plants are being conducted (Rahmann and Seip, 2006; Burke et al., 2009; Lu et al., 2010).

To fight pathogenic organisms, microbiologists and virusologists are researching the effect of flavouring agents in food production (Chiang et al., 2005; Sato et al., 2006; Somolinos et al., 2008; Si et al., 2009; Belletti et al., 2010). Because pigs consume food not only of vegetable origin, but also of animal origin, their diet can include synthetic flavouring agents from the diet of humans. Therefore the objective of this research is to define the effect of flavouring agents upon the level of viability of *S. ransomi* larvae (Rhabditida, Strongyloididae).

Materials and methods

The faeces of pigs were studied to find *S. ransomi* larvae using the Baermann test (Zajac et al., 2011). The material was collected in the summer period in Dnipropetrovsk district, and then transferred to the laboratory in plastic containers at a temperature of 22–24 °C. *S. ransomi* culture is represented by larval stages of freely moving and parasitic forms (Van Wyk and Mayhew, 2013).

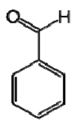
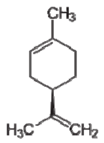
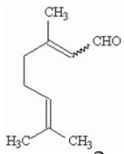
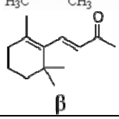
Larvae of first and second stage (freely moving) have a rhabdite-like oesophagus with two bulbuses. Their intestinal cells are not distinguishable. The parasitic form (third stage) is distinguished by a filariform oesophagus with no extensions; the intestine does not have cells which can be clearly distinguished through an optical microscope (Fig. 1).

After cultivation, the liquid with the larvae was put in test tubes (10 ml) by 4 ml and was centrifuged for four minutes at 1500 revolutions a minute. 1 ml of sediments with larvae was evenly weighed and put in 0.1 ml portions in plastic containers with a capacity of 1.5 ml. After that, 1 ml of the substance under research was added to the larval culture (20–40 ind.), which then was kept for 24 hours at a temperature of 22–24 °C. Four concentrations (10, 1, 0.1 и 0.01 g/l) of each of the flavouring agents were used in the experiments (Table 1), with eight replications.

Results

The flavouring agents citral, benzaldehyde exhibited the strongest effect on the viability of *S. ramsomi* larvae (Table 2).

Table 1
Properties and usage of flavouring agents* which were used for establishing the viability level of *S. ransomi* larvae

Name of the substance	Chemical formula	Structural formula	Properties	Content	Usage	
					in food industry	in medicine
Benzaldehyde	C_6H_5CHO		colourless liquid with odour of bitter almond or of apple pips	cores of kernels of bitter almond, apricot kernels, stones of peaches, cherries, black cherries and other stone fruits, leaves of bird cherry tree, pulp of oyster mushroom (*Pleurotus ostreatus*)	as food additive	no data concerning usage
D-limonene (1-methyl-4-isopropenylcyclohexene-1)	$C_{10}H_{16}$		colourless liquid with odour of citrus	many essential oils, including citrus	as food additive	no data concerning usage
Citral (3,7- dimethyl -2,6-octadienal)	$C_{10}H_{16}O$		viscous colourless or bright-yellow fluid with odour of lemon	essential oil of lemon grass (citronella), oil of cubeb, lemon essential oil, eucalyptus and some other essential oils	as food additive	antiseptics anti-inflammatory agent, used to relieve intracranial hypertension
Beta-ionone ((3E)-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-en-2-one)	$C_{13}H_{20}O$		has a pleasant flower odour	some essential oils	as food additive	no data concerning usage

Note: * – Official Journal of the European Union, L 354/46 (2008), Sun (2007), Smirnov (2008).

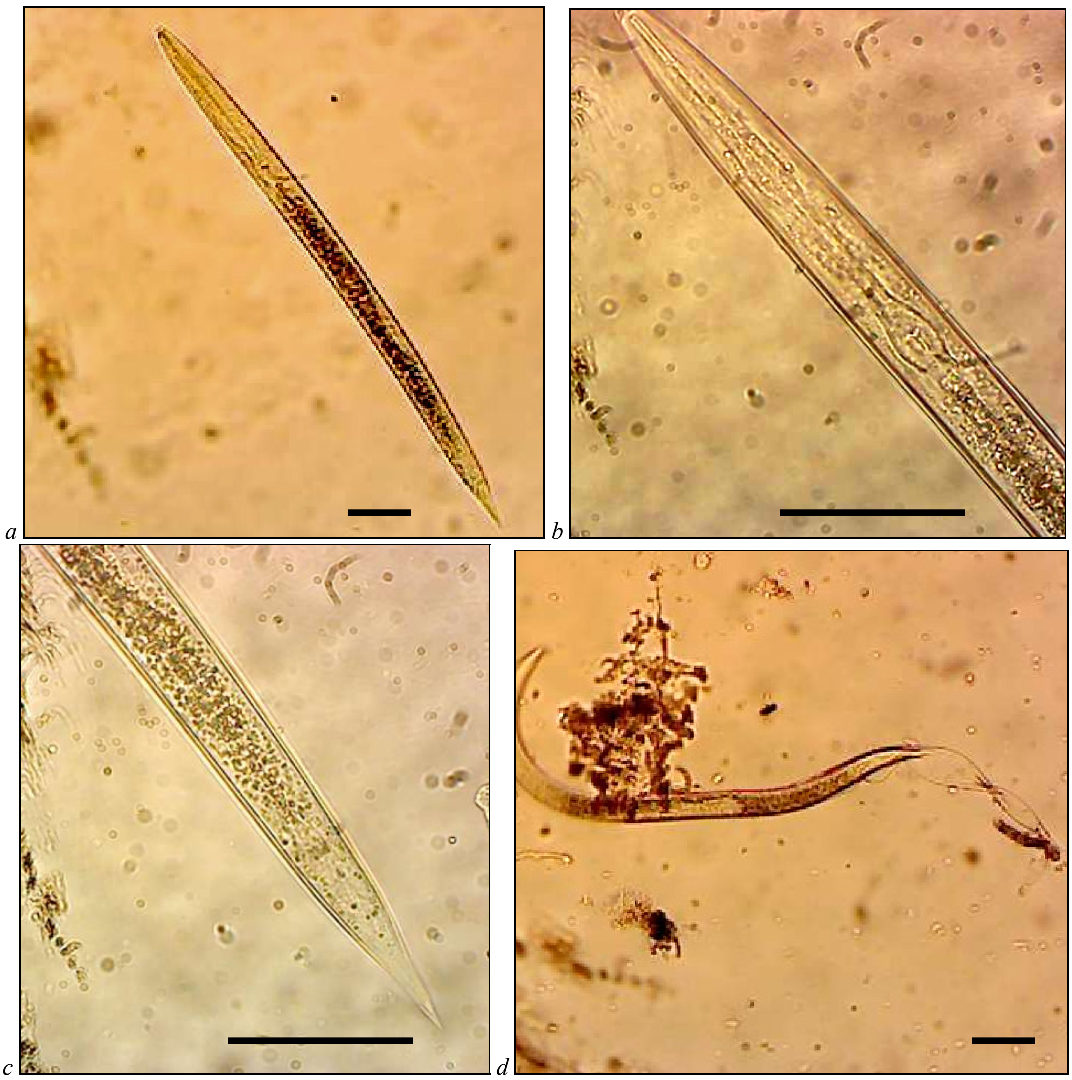


Fig. 1. *S. ransomi* larvae: *a* – noninvasional stage, *b* – filariform oesophagus, *c* – caudal end, *d* – outlet from the cuticle: length of black bar – 50 µm

Table 2
LD_{50} (x ± SD) for *S. ransomi* larvae in laboratory experiment

Название вещества	LD_{50}, мг/л
Benzaldehyde	142 ± 64
D-limonene	–
Citral	97 ± 36
Beta-ionone	–

Note: «–» – the experiment did not achieve death of 50% larvae (needed concentration of more than 10 g/l).

D-limonene and beta-ionone at the studied concentrations (less than 10 g/m) did not affect the viability of *S. ransomi* larvae signifycantly, which shows that they are not useful as nematocidal medicines. The maximum effect upon the nematodes at LD_{90} (Fig. 2*a*, *c*) was shown by benzaldehyde at 685 mg/l. In this case, the larvae of the 100% nematodes studied perished in benzaldehyde solutions with a concentration of 1 and 10 g/l. Thus, this substance can be used for further development of veterinary drugs with anthelmintic effect.

Discussion

Nowadays research is being conducted on the extent of effect of flavouring agents on the viability of agents of infection. Chiang et al. (2005) discovered the antiviral function of a broad spectrum of apigenin, linalool and ursolic acid. These substances are extracted from basil, which is familiar in Chinese medicine as a medicinal plant. The antimicrobial function of these compounds such as linalool and citral is well attested. They are capable of inhibiting the growth of pathogenic microorganisms (Sato et al., 2006; Somolinos et al., 2008; Si et al., 2009; Belletti et al., 2010). Research on the effect of citral on infectious agents also supports the findings from our experiments on the viability of *S. ransomi* larvae. The effect of benzaldehyde and beta-ionone has been insufficiently studied.

For pest control in agriculture a number of authors have suggested using the food flavouring agent cinnamic aldehyde and also acaricidal substances (Knoblauch and Fry, 2011; Na et al., 2011; Shen et al., 2012; Belkind et al., 2013). According to Shen et al. (2012), after using this flavouring agent for 24 hours, LD_{50} for *Psoroptes* was 107 mg/ml. Na et al. (2011) used it against *Dermatophagoides* of birds: LD_{50} after 24 hours was 0.54 mg/ml. This substance was also tested by Cheng et al. (2009) against mosquito larvae: during 24 hours LD_{50} was 40.8 mg/ml (LD_{90} = 81.7 mg/ml) and 46.5 mg/ml (LC_{90} = 83.3 mg/ml) for cinnamic aldehyde and cinnamic acetate, respectively. According to Lee et al. (2008), cinnamic aldehyde, benzaldehyde, linalool, limonene, alpha-Terpineol and other flavouring agents have insecticidal properties against *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera, Curculionidae): LD_{50} at 48 hours exposure was 0.004–0.200 mg/cm^2.

As a fungicidal substance it is advised to use E_{210} (Codex Alimentarius), or benzoic asid, which is also included in other food additives, such as E_{211} – Sodium Benzoate, E_{212} – Potassium Benzoate, E_{213} – Calcium dibenzoate (Beerse et al., 2001; Amborabe et al., 2002; Joshi et al., 2008).

Methylparabene is used against fungi, and also as an antiseptic (Shapiro et al., 2002; Posey et al., 2005; Kromidas et al., 2006; Rebbeck et al., 2006; Ishiwatari et al., 2007; Gopalakrishnan et al., 2012). This substance is also included as a preservative in an insecticide aimed at controlling agricultural pests (Bell, 1990).

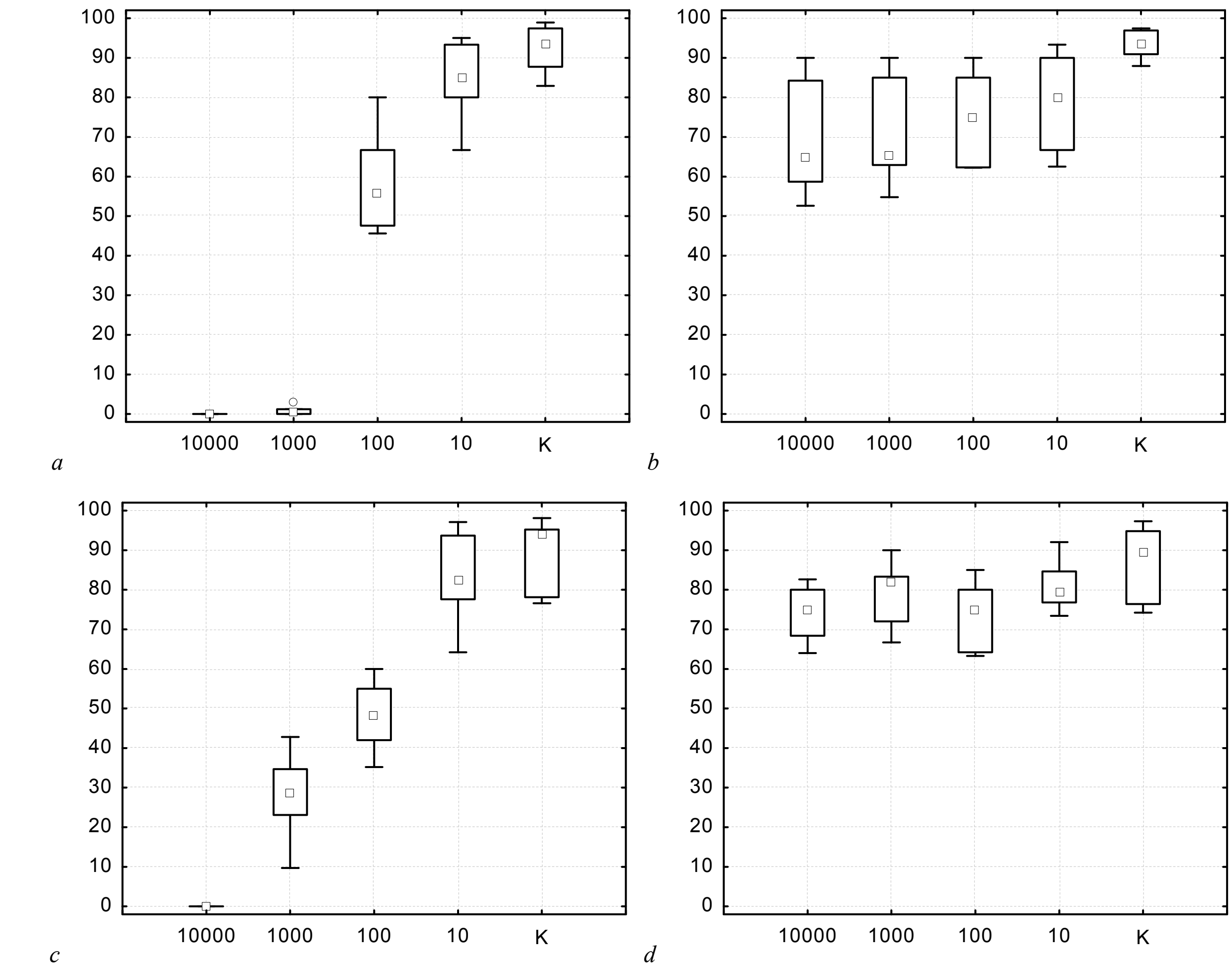


Fig. 2. The effect of benzaldehyde (*a*), D-limonene (*b*), citral (*c*) and beta-ionone (*d*) on the viability of *S. ransomi* larvae: along the axes of abscissa – variant of experiment (mg/l), along the axes of ordinate – viability of *S. ransomi* larvae during 24 hours of laboratory experiment (%); n = 8 for each variant of experiment

The flavouring agent benzaldehyde also shows insecticidal properties. The negative effect of benzaldehyde upon *Galleria mellonella* (Linnaeus, 1758) has been demonstrated. Therefore it is recommended for use in developing new agricultural insecticidal preparations (Ullah et al., 2015). The effect of beta-ionone upon living organisms has received insufficient study. Indeed, the effect of entire groups of flavouring agents upon living organisms requires further studyin general.

Conclusions

We researched the effect of flavouring agents such as benzaldehyde, citral, D-limonene and beta-ionone upon the viability of *S. ransomi*, parasitic larvae on pigs. We determined the minimum values of LD_{50} for benzaldehyde and citral. Experimentally, a death rate of 50% of the tested larvae was not achieved using D-limonene and beta-ionone. Food additives with a pleasant flower odour, which are permitted to be used for food, and which are used as cosmetics, are important for evaluating potential new antihelminthic medical and veterinary preparations.

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