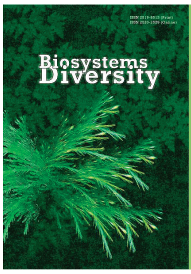

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Nematicidal activity of aqueous tinctures of medicinal plants against larvae of the nematodes *Strongyloides papillosus* and *Haemonchus contortus*

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The study focuses on *in vitro* effect of aqueous tinctures of 48 species of herbaceous, shrub and tree plants on the first-third stage larvae of *Strongyloides papillosus* (Wedl, 1856) and third-stage larvae of *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898. The highest level of the effect was exerted by 3% aqueous tinctures of *Wisteria sinensis* (Sims) DC., *Ailanthus altissima* (Mill.) Swingle, *Laburnum anagyroides* Medik., *Quercus petraea* subsp. *iberica* (Steven ex M. Bieb.) Krassiln., *Ginkgo biloba* L., *Colchicum autumnale* L., *Aristolochia manshuriensis* Kom., *Celastrus scandens* L., *Securigera varia* (L.) Lassen, *Magnolia kobus* DC. Over 90% of the first and second non-invasive stage larvae of *S. papillosus* died at contact with these tinctures. The lowest parameters of LD_{50} were seen for *L. anagyroides*, *Juniperus sabina* L., *C. scandens*, *M. kobus*, *A. manshuriensis*, *Wisteria sinensis* (Sims) DC. and *Securigera varia* (L.) Lassen. Invasive larvae of *S. papillosus* and *H. contortus* were resistant to the effect of all the 48 surveyed species of plants. Third-stage larvae of *H. contortus* remained vital when exposed for 24 h to all the studied concentrations up to 3% aqueous tincture of plants. The results of the experiments and also the analysis of the literature indicate the necessity to continue the survey on nematocidial activity of aqueous tinctures and alcveshol extracts of plants.

Keywords: mortality of larvae; nematocidial activity; aqueous tinctures of medical plants; Nematoda.

Introduction

The health of agricultural animals, including the intensity of their infection with parasites, depends on the conditions of their maintenance. An important aspect regarding their productivity, both during stable maintenance and the grazing period, is the animals' diet (Zazharska et al., 2018). In the pastures, the process of the formation of parasitic fauna is affected by various factors. The most important among the biotic factors are temperature regime, parameters of moisture, mechanical composition of soil, its moisture penetrability, level of salinity, etc. (Boyko, 2008a, 2008b; Yevstafieva et al., 2019). At the same time, none of the separate factors in themselves has a great effect, for it is their mutual impact on parasites which is important. A significant role is played by the species composition of the parasitofauna, relations between all species of the pasture community, including agricultural animals, earthworms, mollusks, Acari and insects – the inhabitants of the pastures' soils and feces of mammals, and also various species of meadow grasses. The impact of invertebrates on the life cycles of the parasites of animals, their distribution and survival in the environment in most cases are studied sufficiently. A relevant aspect is the effect of the preparations or substances of non-synergic origin (Boyko & Brygadyrenko, 2018, 2019a), medicinal and other species of plants on the survival of parasites in the organism of the host in the environment (Boyko & Brygadyrenko, 2019b).

Anthelmintic properties of plants against parasites of agricultural animals are being studied all around the world. Gogoi et al. (2016) evaluated *in vitro* and *in vivo* potential anthelminth actions of extract from leaves of *Caesalpinia bonducella* (L.) against *Syphacia obvelata* (Rud, 1802) (Nematoda) and *Hymenolepis diminuta* (Rudolphi, 1819) (Cestoda): significant anthelmintic action of the extract was seen towards both parasites. In the *in vitro* experiment, the concentration of the extract in the amount of 30 mg/mL caused death to *H. diminuta* after 2.5 ± 0.2 h and *S. obvelata* after 3.6 ± 0.2 h. These studies confirm the fact that the extract from leaves of *C. bonducella* has a significant anthelmintic effect. Decoction of bark of *Oroxylum indicum* (L.) Kurz. (Bignoniaceae) is used in India as a traditional medicine for the treatment of helminthiases of the digestive tract. Deori et al. (2015) studied in vitro and also in vivo anthelminth efficacy of the methanol extract of bark of *O. indicum* against *H. diminuta*; the authors have confirmed that the extract of bark of *O. indicum* has an antiparasitic effect against larvae and adult helminths of *H. diminuta*.

Aqueous and chloroform extracts from coconut, onion, garlic, fig, *Phoenix*, *Cichorium*, pineapple and *Cistus cyprius* were tested against cestodes and trematodes. Abdel-Ghaffar et al. (2010) performed *in vivo* and *in vitro* experiments for anthelminth activity against cestodes of *H. diminuta*, *H. microstoma* Dujardin, 1845, *Taenia taeniaeformis* Batsch, 1786, and also trematodes of *Fasciola hepatica* Linnaeus, 1758 (Trematoda) and *Echinostoma caproni*. In all *in vitro* tests the parasites were killed. The treatment of mice and rats with combination of the extracts from onion and coconut caused death to all the cestodes. The same composition was effective against *E. caproni*. However, it was ineffective against *F. hepatica* in the definitive host. Nonetheless, in *in vitro* experiment, *F. hepatica* trematodes died under the effect of the extracts from this species of plants. Inoculation of fluids of coconut was lethal to *T. taeniaeformis* in naturally-infected cats.

The effects of plants on *H. diminuta* were also studied by Yadav et al. (2010). They evaluated anthelmintic activity of methanol extract of ripe fruits of *Solanum myriacanthum* Dunal with use of *H. diminuta* in albino rats. *Solanum myriacanthum* is a perennial shrub used in folk medicine of one of the Indian tribes for treatment against intestinal helminths. Plant extract exhibited decrease in the amount of produced eggs of helminths, and also number of helminths of *H. diminuta* at all stages of the development in rats depending on the concentration of the extract. The greatest effect by the methanol extract was shown against mature stage of *H. diminuta*. Against this species of parasite, anthelmintic effect was exerted by *Psidium guajava* L. and *Lasia spinosa* (L.)

Thwaites (Temjenmongla et al., 2015). The parasitic nematode *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898 is one the commonest species of helminths of sheep and goats. In Kenya, measures against this parasite are taken using anthelmintic preparations of synthetic origin. Githiori et al. (2003) tested 10 species of plants in the *in vivo* experiment on *H. contortus* of sheep. Among the plants tested on sheep, the most significant decrease in the number of eggs equaling 34% was displayed by bark of *A. anthelmintica* (A. Rich.) Brogn. The rest of the studied species of plants had no significant effect on *H. contortus* of sheep. Thus, *H. contortus* is a nematode which is quite resistant to various factors. Therefore, the objective of our study was the evaluation of *in vitro* nematocidial properties of 48 species of medical plants on nematode larvae of *S. papillosus* and *H. contortus* in different stages.

Material and methods

In the experiment, feces of small ruminants were used. Goats were maintained in the territory of clinical-diagnostic center of the Dnipro State Agrarian-Economic University (Dnipro, Ukraine). The maintenance conditions of goats were satisfactory. In summer the goats grazed, and spent some time on the premises. The animals had free access to food and water. The feces were surveyed for the presence of eggs of nematodes, cestodes, trematodes, larvae of lung nematodes of sheep. The feces were analyzed using McMaster and Baermann tests (Zajac et al., 2011). The cultures of *S. papillosus* were grown in fresh (1–5 h) feces of goats. Exposure of the cultivation of *S. papillosus* and *H. contortus* larvae lasted for 8 days. The larvae were obtained using the Baermann method. For this purpose, 15 g of feces with cultivated larvae were covered by 20 mL of distilled warm (36 °C) water. The exposure for the emergence of larvae from the goats' feces was 2–4 h.

Then, the distilled water with larvae were poured by 4 mL into test tubes and centrifuged for 5 min at 1,500 rpm. Total of 3 mL of supernatant fluid was removed with a pipette. The sediment with larvae (1 mL) was uniformly stirred and put into test tubes (1.5 mL) by 0.1 mL in each. In the experiment, we used five concentrations of aqueous tincture (Table 1) for each of the 48 species of plants. Each variant of the experiment was replicated seven times ($n = 7$). In the control, nematode larvae were kept in aqueous tinctures of plants for 24 h. Then, in the contents of each test tube, the number of live and dead invasive (filarial) and non-invasive (rhabditiform) larvae was counted. The data in the Tables are given in the form of mean ± mean square deviation ($x \pm SD$).

Results

Higher resistance to the influence of the tinctures of medical plants was seen in the invasive nematode larvae of the surveyed species (*S. papillosus* and *H. contortus*). The highest mortality of larvae (Table 1) was observed during exposure to 3% aqueous tinctures of *Wisteria sinensis* (Sims) DC., *Ailanthus altissima* (Mill.) Swingle, *Laburnum anagyroides* Medik., *Quercus petraea* subsp. *iberica* (Steven ex M. Bieb.) Krassiln., *Ginkgo biloba* L., *Colchicum autumnale* L., *Aristolochia manshuriensis* Kom., *Celastrus scandens* L., *Securigera varia* (L.) Lassen, *Magnolia kobus* DC. The percentage of killed specimens was within 90–98%.

Under the effect of 0.75% solutions of *Juniperus sabina* L., *L. anagyroides*, *A. altissima*, *W. sinensis*, *C. scandens*, *A. manshuriensis*, *M. kobus*, *S. varia*, *G. biloba*, the percentage of dead larvae amounted to over 50%, ranging within 50–80%. Lower concentrations of the tinctures of plants exerted no effect against non-invasive larvae of nematodes. Over 24 h of exposure to aqueous solutions of the medical plants in lower concentrations, more than 50% of *S. papillosus* non-invasive larvae and 100% of invasive nematode larvae survived (Table 1).

The lowest LD_{50} parameters were recorded for *L. anagyroides*, *J. sabina*, *C. scandens*, *M. kobus*, *A. manshuriensis*, *W. sinensis*, *S. varia*. Invasive nematode larvae of *S. papillosus* and *H. contortus* were resistant to the effect of even 3% solutions of aqueous tinctures. However, among these two species of nematodes, the more susceptible to the effect were the invasive larvae of *S. papillosus*. The highest effect was exhibited by 3% aqueous tinctures of *M. kobus*, *Q. petraea*, *C. autumnale* and *C. scandens*. During 24 h exposure to 3% tincture of these plants, on average 10–11% of the specimens of *S. papillosus* invasive larvae died (Table 2).

Discussion

Therefore, several species of plants were distinguished which have a negative effect on larvae of *S. papillosus*. And although mostly the larvae of first and second stages of the development died, more detailed understanding of the peculiarities of some of these plants' chemical composition, as well as their use in medicine and veterinary medicine, is required for future experiments against the free-living stages of parasites in the environment.

Wisteria sinensis (Sims) DC. is one of the plants we studied in 3% aqueous tincture with notable nematocidial properties. It also has antioxidant and antibacterial properties. *Wisteria sinensis* contains large amounts of polyphenols, saponins, flavons and lectins. Keskin et al. (2019) consider that having such properties the plant can be used for treatment of rheumatoid arthritis, diseases of the stomach and the mammary gland. Jiang et al. (2009) demonstrated the inhibiting effect of *W. sinensis* on fungi and bacteria (these authors used acetyl extract from leaves of *W. sinensis*). These experiments, according to the authors, require further research in the sphere of the development of agricultural chemicals. Mohamed et al. (2019) also performed research using fat-free aqueous 80% methanol extract from leaves of *W. sinensis*. For the first time they report that this extracts has notable cytotoxic activity towards Hep-G2 line of tumour cells, in addition to antioxidant activity. Our studies, in turn, also indicate the need for further survey of nematocidial properties of this plant using other methods of extracting the active substances.

Ailanthus altissima (Mill.) Swingle is used in traditional Chinese medicine for the treatment against colds and stomach diseases. *Ailanthus altissima* contains essential oils of different parts of the plant: roots, stems, leaves, flowers and ripe fruits. Ayeb-Zakhama et al. (2014) extracted 69 compounds. They were represented by 91.0–97.2% of all oil composition. Essential oil from the root contains large amounts of aldehydes (22.6%), whereas the leaves mostly contain oxygen-enriched sesquiterpenes (42.1%). Essential oil was rich in derivative of apocarotenoids, hexahydrofarnesyl acetate (58.0%), and the oil obtained from stems was characterized by sesquiterpene hydrocarbons (54.1%), mainly β-caryophyllene (18.9%). These authors also report phytotoxic effect of *A. altissima*.

Albouchi et al. (2013) also studied the chemical composition of *A. altissima*, its antioxidant, antimicrobial and phytotoxic activity. They performed a complex study of volatile oils and phenol components of leaves of *A. altissima*. In the experiment, methanol extracts of leaves and their hydrodistilled remains were used. The essential oils from leaves were found to contain over hundred constituents. Most of them are represented by non-terpene compounds (tetradecanol, heneicosane, tricosane and docosane), and also sesquiterpene hydrocarbons (α-curcumene and α-gurjunene). Methanol extracts from leaves contain higher amount of phenols, while extracts from hydrodistilled remains – high total content of flavonoids. Most commonly found phenol compounds were gallic acid, chlorogenic acid, HHDP-galloylglucose, epicatechin, rutin, hyperoside and quercetin-3-galloylglucoside. Both extracts exerted strong antioxidant activity depending on the concentration. These extracts are efficient against Gram-positive bacteria (Albouchi et al., 2013) and inactive towards Gram-negative bacteria and *Candida albicans*. They also exhibited high inhibiting effect on germination and growth of rots of wild carrot *Daucus carota* L. Phenol components of extracts from leaves of *A. altissima* (methanol and aqueous extracts), their antibacterial and anti-fungal properties were studied by Poljuha et al. (2017). Methanol extract contained more phenol, phlavonoid and non-phlavonoid compounds, and also had more notable antioxidant activity than aqueous extracts. Acetone extract from leaves displayed activity against *Escherichia coli*. Acetone, methanol and dichloromethane extracts had high activity towards *Candida albicans* (Poljuha et al., 2017).

Ailanthus altissima contains proteins, flavonoids, alkaloids, quassinoids, terpinyl coumarins, tetracyclic triterpenoids, fatty acids, essential oils and many other active compounds. Al-Snafi (2015) report antibacterial, antiviral, antioxidant, cytotoxic, anti-diarrhea, anti-inflammatory, antipyretic, anesthetic, antihistamine, antiparasitic, repellent, anti-progesterone activities of this medical plant. Bary et al. (1987) and also Okunade et al. (2003) studied *in vitro* antiplasmodial activity of *A. altissima*.

Studies were conducted for the *in vitro* effect of bark of *A. altissima* on *Psoroptes cuniculi* (Delafond, 1859) and *Sarcoptes scabiei* (Linnaeus, 1758). In the concentrations of 1.00, 0.50 and 0.25 g/mL, 100% of *S. scabiei* died over 7 h exposure. The concentrations of 1.0 and 0.5 g/mL killed all the tested *P. cuniculi* (Gu et al., 2014). Repellent and fumigative effects were exerted by essential oil of *A. altissima* (Lü et al., 2011). Also, mollusicidal effect of *A. altissima* was confirmed (Jiang et al., 2013). In their studies, El-Rigal et al. (2006) demonstrated antiparasitic and repellent effects of the extract of chlorophorm of stem bark of *A. altissima*. The scientists revealed antischistosomal and hepatoprotective effects. Also, during the use of this extract, they observed decrease in the number of helminths in mice. Methanol extracts of different parts of plant *A. altissima* were tested against root-knot nematode *Meloidogyne javanica* (Caboni et al., 2012). According to the results of our studies, *A. altissima* has also nematocidial properties against *S. papillosus* nematode larvae in the first and second stages of the development.

Laburnum anagyroides Medik. is consumed by larvae of some species of lepidopterans as food. *Laburnum anagyroides* is considered a poisonous plant (Forrester, 1979). The plant's main toxin is cytosine. The symptoms of poisoning can manifest in strong somnolence, nausea, vomiting, diarrhea, spasmodic movements and even coma. Perhaps, toxic properties of this poisonous plant also negatively affected the larvae of the first and second stages of *S. papillosus* nematodes.

Table 1
Mortality (%) of non-invasive larvae of *S. papillosus* $L_{1–2}$ during 24 h laboratory experiment (x ± SD, n = 7) exposed to aqueous tinctures of leaves from 48 species of plants

Family	Species	Mortality of nematode larvae in 3.0% plant solution, %	Mortality of nematode larvae in 0.75% plant solution, %	Mortality of nematode larvae in 0.188% plant solution, %	Mortality of nematode larvae in 0.047% plant solution, %	Mortality of nematode larvae in 0.012% plant solution, %	Mortality of nematode larvae in control, %	LD_{50}, %
Anacardiaceae	*Cotinus coggygria* Scop.	28.2 ± 1.9	7.8 ± 0.9	6.1 ± 1.4	5.7 ± 1.6	6.4 ± 0.6	3.7 ± 2.1	–
–“–	*Rhus trilobata* Nutt.	48.5 ± 5.4	32.2 ± 3.2	14.3 ± 1.7	10.8 ± 1.1	10.3 ± 0.3	11.9 ± 1.6	–
–“–	*Rhus typhina* L.	19.4 ± 2.2	10.0 ± 1.0	5.9 ± 0.4	5.1 ± 2.3	3.7 ± 1.2	3.2 ± 1.2	–
–“–	*Toxicodendron orientale* Greene	79.3 ± 3.2	47.7 ± 3.1	14.0 ± 2.8	7.4 ± 2.2	8.6 ± 1.6	11.9 ± 1.6	0.90
Araliaceae	*Aralia elata* (Miq.) Seem.	65.7 ± 1.7	51.8 ± 3.8	20.1 ± 1.6	6.1 ± 3.1	4.3 ± 1.4	3.2 ± 1.2	0.75
–“–	*Hedera helix* L.	39.7 ± 6.7	12.0 ± 3.1	5.9 ± 1.8	3.9 ± 2.0	4.1 ± 1.4	3.7 ± 2.1	–
Asparagaceae	*Polygonatum multiflorum* (L.) All.	23.7 ± 5.6	12.0 ± 3.1	5.9 ± 1.8	3.9 ± 2.0	4.1 ± 1.4	3.7 ± 2.1	–
Berberidaceae	*Berberis vulgaris* L.	46.9 ± 4.9	23.6 ± 1.9	15.9 ± 3.4	13.4 ± 3.4	8.0 ± 1.7	7.1 ± 2.8	–
–“–	*Mahonia aquifolium (Pursh)* Nutt.	35.4 ± 8.2	15.5 ± 1.1	13.3 ± 1.4	8.7 ± 1.8	8.2 ± 0.7	7.1 ± 2.8	–
Bignoniaceae	*Campsis radicans* (L.) Seem.	48.9 ± 3.4	19.4 ± 2.4	11.5 ± 1.9	11.4 ± 2.5	9.1 ± 1.2	11.1 ± 0.9	–
–“–	*Catalpa fargesii* Bureau	27.3 ± 2.6	21.9 ± 0.8	11.0 ± 0.8	10.5 ± 2.3	10.4 ± 1.4	11.9 ± 1.6	–
Calycanthaceae	*Chimonanthus praecox* (L.) Link	25.7 ± 1.9	14.8 ± 2.6	6.5 ± 1.4	3.1 ± 1.4	3.0 ± 1.3	3.7 ± 2.1	–
Celastraceae	*Aristolochia manshuriensis* Kom.	94.6 ± 1.7	70.5 ± 3.9	46.2 ± 2.6	13.8 ± 3.0	7.6 ± 1.4	7.1 ± 2.8	0.24
–“–	*Celastrus scandens* L.	92.5 ± 1.4	70.0 ± 2.7	47.1 ± 3.0	13.9 ± 2.3	8.6 ± 1.1	7.1 ± 2.8	0.23
Colchicaceae	*Colchicum autumnale* L.	92.8 ± 2.2	49.1 ± 2.9	15.7 ± 2.9	13.5 ± 2.2	8.0 ± 1.0	7.1 ± 2.8	0.80
Cupressaceae	*Chamaecyparis lawsoniana* (A. Murray bis) Parl.	72.3 ± 5.9	46.7 ± 1.9	10.0 ± 2.0	8.5 ± 3.1	6.3 ± 2.7	3.2 ± 1.2	1.08
–“–	*Juniperus sabina* L.	85.7 ± 2.0	71.5 ± 3.1	47.3 ± 3.6	17.9 ± 2.5	10.7 ± 1.1	11.9 ± 1.6	0.22
Dennstaedtiaceae	*Pteridium aquilinum* (L.) Kuhn	61.5 ± 1.8	37.6 ± 1.7	22.8 ± 1.7	16.6 ± 2.7	10.5 ± 1.2	11.9 ± 1.6	1.93
Eucommiaceae	Eucommia ulmoides Oliv.	50.7 ± 12.5	21.4 ± 4.2	6.3 ± 1.7	2.9 ± 1.3	3.3 ± 2.1	3.7 ± 2.1	2.94
Fabaceae	*Genista tanaitica* P. A. Smirn.	41.0 ± 4.2	20.8 ± 1.8	10.1 ± 2.3	6.4 ± 2.6	5.4 ± 2.7	3.2 ± 1.2	–
–“–	*Laburnum anagyroides* Medik.	90.7 ± 1.6	66.7 ± 3.5	48.9 ± 4.4	21.5 ± 2.2	7.8 ± 1.2	7.1 ± 2.8	0.19
–“–	*Securigera varia* (L.) Lassen	91.7 ± 1.3	79.2 ± 5.9	15.4 ± 4.8	10.1 ± 1.2	7.7 ± 1.0	7.1 ± 2.8	0.48
–“–	*Styphnolobium japonicum* (L.) Schott	63.7 ± 3.2	31.4 ± 7.1	15.7 ± 4.7	6.2 ± 2.9	3.9 ± 1.0	3.2 ± 1.2	2.07
–“–	*Wisteria sinensis* (Sims) DC.	90.0 ± 3.4	80.3 ± 9.4	40.2 ± 4.1	13.3 ± 1.8	6.8 ± 1.1	7.1 ± 2.8	0.31
Fagaceae	*Quercus castaneifolia* C. A. Mey.	50.6 ± 5.1	23.0 ± 4.1	14.0 ± 2.2	13.4 ± 2.4	7.4 ± 1.3	7.1 ± 2.8	2.96
–“–	*Quercus petraea subsp. iberica* (Steven ex M.Bieb.) Krassiln.	94.7 ± 1.3	48.0 ± 2.7	17.6 ± 1.4	10.4 ± 1.0	8.2 ± 2.1	7.1 ± 2.8	0.84
Geraniaceae	*Geranium sanguineum* L.	16.3 ± 1.9	12.0 ± 1.8	9.9 ± 1.6	9.7 ± 1.3	9.5 ± 1.8	11.9 ± 1.6	–
Ginkgoaceae	*Ginkgo biloba* L.	92.3 ± 2.9	49.5 ± 3.3	17.2 ± 2.1	10.1 ± 1.6	7.9 ± 1.3	7.1 ± 2.8	0.79
Lamiaceae	*Callicarpa bodinieri* H. Lev.	56.1 ± 1.9	15.1 ± 3.6	8.2 ± 5.5	4.6 ± 2.0	4.7 ± 2.1	3.7 ± 2.1	2.67
–“–	*Nepeta mussinii* Spreng.	50.7 ± 4.0	19.4 ± 4.6	6.3 ± 1.9	3.6 ± 1.7	3.8 ± 1.9	3.7 ± 2.1	2.95
–“–	*Salvia officinalis* L.	26.1 ± 1.8	12.9 ± 1.4	5.8 ± 0.9	2.7 ± 1.3	2.6 ± 1.1	3.7 ± 2.1	–
–“–	*Vitex agnus-castus* L.	30.2 ± 1.9	19.1 ± 1.7	10.0 ± 1.2	10.2 ± 1.3	9.3 ± 1.5	11.9 ± 1.6	–
–“–	*Vitex negundo* L.	62.0 ± 3.0	44.3 ± 1.4	11.9 ± 1.6	9.1 ± 4.2	6.7 ± 3.0	3.2 ± 1.2	1.50
Magnoliaceae	*Liriodendron tulipifera* L.	36.4 ± 3.6	22.4 ± 1.1	10.8 ± 0.6	10.4 ± 2.3	9.5 ± 1.3	10.9 ± 1.2	–
–“–	*Magnolia kobus* DC.	97.6 ± 2.2	72.9 ± 4.3	44.5 ± 1.9	14.1 ± 3.2	7.6 ± 1.3	7.1 ± 2.8	0.26
Moraceae	*Maclura pomifera* (Raf.) Schneid.	52.6 ± 14.0	23.6 ± 4.0	6.2 ± 1.3	4.4 ± 2.2	3.8 ± 1.1	3.2 ± 1.2	2.80
Phyllanthaceae	*Leptopus chinensis* (Bunge) Pojark.	44.7 ± 1.2	24.2 ± 2.6	8.4 ± 0.6	6.2 ± 2.3	4.1 ± 1.2	3.2 ± 1.2	–
Pinaceae	*Pseudotsuga menziesii* (Mirb.) Franco	60.0 ± 1.7	35.9 ± 6.7	12.4 ± 4.1	6.7 ± 3.4	4.3 ± 1.9	3.2 ± 1.2	2.06
Ranunculaceae	*Clematis flammula* L.	47.8 ± 4.2	22.9 ± 1.5	16.0 ± 2.5	13.5 ± 3.4	7.2 ± 1.5	7.1 ± 2.8	–
Rosaceae	*Prunus dulcis* (Mill.) D. A. Webb	41.6 ± 2.4	11.7 ± 2.7	8.2 ± 2.1	5.6 ± 1.9	5.5 ± 1.4	3.7 ± 2.1	–
–“–	*Prunus laurocerasus* L.	70.8 ± 1.7	28.1 ± 2.0	15.7 ± 2.4	10.9 ± 1.9	10.2 ± 1.0	11.9 ± 1.6	1.91
Rutaceae	*Dictamnus albus* L.	34.9 ± 4.6	23.1 ± 3.3	11.8 ± 2.9	3.0 ± 1.8	2.7 ± 1.1	3.7 ± 2.1	–
–“–	*Phellodendron amurense* Rupr.	64.5 ± 6.0	48.7 ± 2.8	20.8± 2.6	5.8 ± 2.3	4.1 ± 0.9	3.2 ± 1.2	0.90
–“–	*Ptelea trifoliata* L.	59.0 ± 8.6	20.5 ± 1.7	11.1 ± 2.4	10.6 ± 1.8	10.4 ± 1.4	11.9 ± 1.6	2.47
Simaroubaceae	*Ailanthus altissima* (Mill.) Swingle	90.0 ± 3.3	50.7 ± 4.0	18.6 ± 2.5	14.0 ± 2.3	7.4 ± 1.2	7.1 ± 2.8	0.75
Tamaricaceae	*Tamarix elongata* Ledeb.	36.0 ± 5.4	18.4 ± 2.7	5.6 ± 1.8	3.9 ± 2.0	4.1 ± 1.8	3.7 ± 2.1	–
Taxaceae	*Cephalotaxus harringtonia* (Knight ex J. Forbes) K. Koch	63.6 ± 5.1	45.1 ± 2.6	10.3 ± 1.7	7.2 ± 1.3	6.6 ± 0.8	3.2 ± 1.2	1.32
Vitaceae	*Parthenocissus tricuspidata* (Siebold & Zucc.) Planch.	47.6 ± 4.5	25.9 ± 4.8	13.3 ± 2.5	11.2 ± 2.4	7.4 ± 1.4	7.1 ± 2.8	–

Table 2
Mortality (%) of larvae of *S. papillosus* L_3 during 24 h laboratory experiment (x ± SD, n = 7) exposed to aqueous tinctures of leaves of the plants

Family	Species	Mortality of nematode larvae in 3.0% plant solution, %
Anacardiaceae	*Toxicodendron orientale* Greene	0.6 ± 1.3
Berberidaceae	*Berberis vulgaris* L.	1.3 ± 2.9
Celastraceae	*Aristolochia manshuriensis* Kom.	9.0 ± 7.0
Celastraceae	*Celastrus scandens* L.	10.1 ± 8.1
Colchicaceae	*Colchicum autumnale* L.	10.1 ± 7.6
Cupressaceae	*Juniperus sabina* L.	6.6 ± 7.0
Fabaceae	*Laburnum anagyroides* Medik.	9.3 ± 7.0
Fabaceae	*Securigera varia* (L.) Lassen	6.1 ± 6.7
Fabaceae	*Wisteria sinensis* (Sims) DC.	9.5 ± 7.5
Fagaceae	*Quercus petraea subsp. iberica* (Steven ex M.Bieb.) Krassiln.	10.4 ± 5.8
Ginkgoaceae	*Ginkgo biloba* L.	7.2 ± 7.9
Lamiaceae	*Securigera varia* (L.) Lassen	1.7 ± 3.7
Lamiaceae	*Vitex negundo* L.	0.8 ± 1.7
Magnoliaceae	*Magnolia kobus* DC.	11.0 ± 9.1
Simaroubaceae	*Ailanthus altissima* (Mill.) Swingle	3.9 ± 4.0
Vitaceae	*Parthenocissus tricuspidata* (Siebold & Zucc.) Planch.	1.2 ± 2.7

Note: for the rest of the concentrations (0.750%, 0.188%, 0.047%, 0.012%) of aqueous tinctures of these species of plants the mortality of invasive larvae of *S. papillosus* L_3 was not observed; for all the species indicated in Table 1, but absent in Table 2, mortality of invasive larvae of *S. papillosus* L_3 exposed to the aqueous tinctures of the concentration of 3.00% and less was also not seen.

Aristolochia manshuriensis Kom. is a Chinese medicinal plant which in certain concentrations is toxic (Zhu, 2002; Hu et al., 2004; Wu et al., 2016).

In nature, *Celastrus scandens* L. is consumed by birds, though its fruits are also poisonous (Plant Finder. Missouri Botanical Garden). Our studies also indicate high mortality of *S. papillosus* larvae of the first and second stages of the development exposed for 24 h to 3% solutions of *C. scandens*.

Leaves of *Ginkgo biloba* L. have a unique chemical composition. Extract of leaves of this medical plant includes over 40 biologically active ingredients. It contains three main groups of substances. The first group is comprised of terpene trilactones, the second of bioflavonoids; the third group is represented by proanthocyanidins or condensed tannins, organic acids (benzoic acid and its derivatives), and also polyphenols, ginkgolic acids, nitrogene bases (thymine), amino acids (asparagines), wax, catechins, steroids, cardanols and other substances (Committee on Herbal Medicinal Products). Long-chain phenols of *G. biloba* have antimicrobial properties. Bilobol and cardanol are active against *Staphylococcus aureus*. Anacardic acid inhibits growth of *Streptococcus pyogenes*, *Staphylosoccus aureus*, *Neisseria gonorrhoeae*, *Bacillus anthracis*, and also has anthelminth properties. Leaves also contain superoxide dismutase enzyme which has antioxidant properties (Zuzuk et al., 2001). Flavoglycosides of *G. biloba* have high antioxidant activity, and terpenes have an anti-inflammatory effect and improve energy methabolism of the brain. Preparations of this plant contribute to improvement of capillary blood circulation, and also can inhibit the development of metastases from malignant tumours and prevent formation of thrombi (Mohanta et al., 2014). Maltas et al. (2011), in their experiments, studied antioxidant property and total content of phenols in the extracts of *G. biloba*. Methanol extract exerted high antioxidant activity associated with high content of phenol. The authors also analyzed the content of fatty acids of methanol and acetone extracts from *G. biloba*. They presume that *G. biloba* grown in Turkey can be an important source of natural antioxidant.

Traditional Korean medicine broadly uses flower buds of *Magnolia kobus* DC. for treating various diseases (Cho et al., 2015). Epicuticular leaf waxes of *G. biloba* and *M. grandiflora* contain homologic series of carbohydrates, complex waxy ethers, complex ethers benzyl acyls, aldehydes, primary alcohols and fatty acids (Gülz et al., 1992). As a result of our studies, on average 98%. the first and second stage larvae of nematodes exposed for 24 h died. Thus, the results of the studies mentioned above and also our experiments indicate the necessity of further surveys in this area.

Conclusion

Invasive larvae of *S. papillosus* and *H. contortus* in *in vitro* experiments were resistant to 3% aqueous tincture of the studied plants. Only the larvae of the third (invasive) stage of *S. papillosus* died at 24 h exposure to *M. kobus*, *Q. petraea*, *C. autumnale* and *C. scandens* on average (10–11%). In *in vitro* experiments, the most resistant to the influence of the studied plants were the third stage larvae of *H. contortus*. Over 95% of larvae of this nematode species remained vital after exposure to aqueous tinctures of the plants. Larvae of *S. papillosus* in first and second non-invasive stages were most sensitive to the effects of tinctures of *W. sinensis*, *A. altissima*, *L. anagyroides*, *Q. petraea* subsp. *iberica*, *G. biloba*, *C. autumnale*, *A. manshuriensis*, *C. scandens*, *S. varia*, *M. kobus*. Mortality of larvae of these stages over 24 h accounted for 90%. The lowest LD_{50} were seen during the impacts of *L. anagyroides*, *J. sabina*, *C. scandens*, *M. kobus*, *A. manshuriensis*, *W. sinensis*, *S. varia*, indicating their notable nematocidial property towards *S. papillosus* larvae of the first and second stages.

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