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RESEARCH ARTICLE

Nematicidal activity of aqueous tinctures of plants against larvae of the nematode *Strongyloides papillosus*

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ABSTRACT

This research was undertaken to evaluate the nematicidal activity of various concentrations of aqueous tinctures of 80 plant species towards L_{1-2} of *S. papillosus*. For the experiment with larvae of *S. papillosus*, there were used 0.19%, 0.75% and 3.00% aqueous tinctures of plants. Out of 80 tested species, nematicidal activity against L_{1-2} of *S. papillosus* was displayed by 20 plants. The greatest activity (LC_{50} = 0.060–0.069%) towards larvae of *S. papillosus* was exerted by *Teucrium polium*, *Achillea millefolium*, *Genista tinctoria* and *Ulmus laevis*. Less expressed nematicidal activity (LC_{50} = 0.070–0.079%) was recorded for *Thalictrum minus*, *Stachys recta*, *Falcaria vulgaris*, *Lavatera thuringiaca*. Even lower effect (LC_{50} = 0.080–0.089%) was shown by aqueous tinctures of *Mentha × piperita*, *Achillea millefolium*, *Salvia nutans*, *Eryngium campestre* and *Cerasus fruticosa*. The following plants could be arranged in declining order of effectiveness of nematicidal activity (LC_{50} = 0.090–0.165%) *Malus sylvestris*, *Tragopogon orientalis*, *Erigeron annuus*, *Grindelia squarrosa*, *Urtica dioica*, *Daucus carota*, *Medicago sativa*, *Carduus acanthoides*, *Ulmus minor* and *Hieracium umbellatum*. A far weaker effect on the nematodes was displayed by *Bromopsis inermis* and *Tragopogon podolicus*. Aqueous tinctures of 60 other studied species of plants exhibited low nematicidal activity in 3.00% aqueous tincture, while in 0.19% and 0.75% aqueous tinctures, no nematicidal activity was seen. The results of the research suggest that in the conditions of natural ecosystems, some species of plants of the Apiaceae, Asteraceae, Fabaceae, Lamiaceae, Malvaceae, Rosaceae, Ulmaceae and Urticaceae families could reduce vitality of free-living L_{1-2} larvae of *S. papillosus*.

Keywords: Strongylidae; Rhabditida; mortality; gastrointestinal helminthiases; anthelmintic preparations.

INTRODUCTION

Every year, farms experience large losses from outbreaks of helminthiases. Among ruminant nematodiases, gastro-intestinal helminthiases are quite frequent (MatYusof *et al.*, 2016; Rupa & Portugaliza, 2016; Zvinorova *et al.*, 2016; Chaudhary *et al.*, 2017; de Almeida *et al.*, 2018; Gupta *et al.*, 2019; Lambacher *et al.*, 2019; Puspitasari *et al.*, 2019). Losses from these diseases may be inflicted not only by the death of animals, but also by decrease in the amount of dairy or meat products and reduction of their quality (Moreno & Lanusse, 2017; Swai & Wilson, 2017; Bellet *et al.*, 2018; Fthenakis & Papadopoulos, 2018; Rashid *et al.*, 2018). Economic losses also occur due to the costs associated with use of synthetic anthelmintic preparations. At the same time, frequent application of such preparations may not provide the necessary positive effect due to the gradual development by the parasites of resistance to anthelmintic preparations (Lyndal-Murphy *et al.*, 2014; Dyary, 2015; Gárcia *et al.*, 2016; Kotze & Prichard, 2016; Singh *et al.*, 2016; Ploeger & Everts, 2018; Sangster *et al.*, 2018; Baiak *et al.*, 2019; Kelleher *et al.*, 2020). Therefore, the search of alternative treatment of helmianthiases without synthetic anthelmintic preparations is a promising direction of studies on host animals and their parasites.

One of the directions of combating nematodoses is the use of medicinal plants (Ayaz *et al.*, 2018; Oliveira Santos *et al.*, 2019; Swargiary *et al.*, 2020). They are used in both *in vitro* (De Paula Carlis *et al.*, 2019; Esteban-Ballesteros *et al.*, 2019; Maestrini *et al.*, 2019; Piza *et al.*, 2019; Boyko & Brygadyrenko, 2018, 2019, 2020), and *in vivo* experiments (Sastya *et al.*, 2018; Alowanou *et al.*, 2019). Efficiency of plant extracts depends on the various conditions of the experiments. Most often alcoholic or hydro-alcoholic extracts are effective against nematodes (Eguale *et al.*, 2007; Olmedo-Juárez *et al.*, 2017; Tsehayneh & Melaku, 2019). Quite often the published

data reports the anthelmintic properties of aqueous plant tinctures (Khan *et al.*, 2018; Nouri *et al.*, 2019; Imani-Baran *et al.*, 2020).

Nematodes of the genus *Strongyloides* Grassi, 1879 have several stages of development. Larvae of first-second stages (rhabditiformlarvae) live in the external environment. Their life cycle comprises two consecutive ways of development in the environment. In the first case second-stage larvae moult and transform into infective larvae (filariform). The second course of the development leads to formation of free-living females and males in the environment, which are able to produce a new generation. The type of development depends on many factors. The most important one is temperature. For example, infective larvae (L_3) develop faster in lower temperatures, while free-living generations emerge in higher temperatures (Premvati, 1963; Nwaorgu, 1983; Shiwaku *et al.*, 1988; Viney, 1996, 1999). Numerous experiments related to the struggle against infective larvae are performed all around the globe. However, the control of L_{1-2}in the environment is also important in order to combat strongyloidiasis.

Therefore, the objective of this study was to assess the *in vitro* effect of aqueous tinctures of plants collected in natural conditions in the south of Ukraine on L_{1-2} of *Strongyloides papillosus* (Wedl, 1856).

MATERIAL AND METHODS

Collection and Identification of the Medicinal Plants

Above-ground parts of medicinal plants were collected in the territory of Regional Landscape Park Samarsky Bor in Novomoskovsk district of Dnipropetrovsk Oblast (Ukraine). The zones of collection of plants were at the distance of over 3 km from industrial enterprises, ploughed fields and major highways. Samples from a total of 80 species of plants were identified and deposited in the Botanical Herbarium of Dnipro National University (Table 1).

Preparation of the Tincture

Tinctures were obtained during heating of 0.5 g of dry fragmented parts of plants (leaves, shoots, inflorescences, stigmas, bark – Table 1) in 150.55 g of distilled water (90°C) over 15 min in the water bath. The obtained tincture was cooled at room temperature to 25°C. Then the obtained tincture was percolated.

Dilution of the Samples

In the experiment three concentrations of aqueous tincture of each of the 80 species of plants were used: 3.00%, 0.75%, and 0.19%. Solution I was obtained by adding 150.55 g of distilled water to 5.0 g of dry fragmented parts of plants. To obtain solution II, 10 g of solution and 30.00 g of distilled water were used. Solution III was prepared by mixing 10 g of solution II and 29.47 g of distilled water.

Larval Cultivation

Feces were collected from naturally infected goats of the Clinical-Diagnostic Center of the Faculty of Veterinary Medicine of Dnipro State Agrarian-Economic University (Ukraine). Analysis of feces for presence of eggs of nematodes was performed using the McMaster technique. Nematode larvae were cultivated in feces for 3 days in the conditions of moistening in the temperature of 28°C. L_{1-2} were extracted using the Baermann test (Zajac *et al.*, 2011). Exposure for the exit of the larvae from the feces lasted for 2–4 h.

Larval Survival Test

A total of 4 mL of water with larvae was poured in test tubes and centrifuged during 4 minutes at 1,500 rpm. Supernatant fluid (3 mL) was removed with a pipette. The sediment with larvae (1 mL) was uniformly mixed and 0.1 mL (60–110 larvae/ 0.1 mL) was put into test tube (1.5 mL). Then, aqueous tinctures of plants were added – solutions I, II and III, 1.4 mL of each, to obtain respectively 3.00%, 0.75% and 0.19% of aqueous tinctures of plants in experimental test tubes. In each variant of the experiment, the surveys were performed in seven-fold replication (n = 7). Nematode larvae were kept in aqueous tinctures of plants and the control (distilled water) for 24 h. Then, in the contents of each test tube, live and dead L_{1-2} were counted under the microscope at 100x magnification.

Statistical Analysis of the Data

The statistical analysis of the results was performed through the set of Statistica 8.0 (StatSoft Inc., USA). For each set of 7 experiments, mean and standard deviations (x ± SD) were calculated for each set of 7 experiments. Reliability of differences of mortality of larvae in the control and aqueous tincture of plant was calculated using ANOVA. In Figure 1 and 2, the small square in the centre corresponds to the median, the lower and upper edge of the large rectangle corresponds to first and third quartiles, respectively, the vertical segments, directed upward and downward from the rectangles, correspond to minimum and maximum values.

RESULTS

The experiment revealed that aqueous tinctures of 20 (3%) of the tested plants exhibited notable nematicidal activity towards L_{1-2} of *S. papillosus* (Table 1). Over 90% of mortality of first and second stage larvae of *S. papillosus* were observed during the influence of aqueous tinctures of *E. campestre*, *F. vulgaris*, *A. millefolium*, *E. annuus*, *G. squarrosa*, *T. orientalis*, *T. podolicus*, *G. tinctoria*, *M. sativa*, *M.* × *piperita*, *S. nutans*, *S. recta*, *T. polium*, *L. thuringiaca*, *T. minus*, *C. fruticosa*, *M. sylvestris*, *U. laevis*, *U. minor*, *U. dioica*. Less than 50% of mortality of first and second stage larvae of *S. papillosus* was found using aqueous tinctures of 26 species of plants.

Aqueous tinctures of plants of the Apiaceae family (Figure 1a–c) exhibited good results against rhabditiform larvae (L_{1-2}) of *S. papillosus*. The most effective plants of this family were *E. campestre* and *F. vulgaris*. Exposure to plants of the Asteraceae family (Figure 1d–j) killed over 80% of L_{1-2} larvae of *S. papillosus* using all the tested concentrations of aqueous tinctures of *A. millefolium* L., *E. annuus* and *T. orientalis*. Aqueous tinctures of low concentrations of the tested species of Fabaceae family *in vitro* displayed no expressed nematicidal properties against larvae of *S. papillosus* (Figure 1k, l).

Aqueous tinctures of four species of the Lamiaceae family (Figure 2a–d) – *M.* × *piperita*, *S. nutans*, *S. recta* and *T. polium* – caused death of 80% of nematode larvae in all three studied concentrations. Aqueous tincture of *L. thuringiaca* (Malvaceae) was also effective (Figure 2e) even in the lowest of the tested concentrations (0.19%).

Tinctures of *B. inermis* (Poaceae) and *T. minus* (Ranunculaceae) (Figure 2f, g) in the concentration of 0.19% killed less than 80% of *S. papillosus* rhabditiform larvae. Plants of the Rosaceae (Figure 2h–i) and Ulmaceae families (Figure 2j–l) had different efficiency against larvae: higher activity in all the studied concentrations was exhibited by *C. fruticosa* (Rosaceae), *U. laevis* (Ulmaceae). Aqueous tincture of *U. dioica*

Table 1. Mortality (%) of rhabditiform larvae (L_{1-2}) of *S. papillosus* exposed to aqueous tinctures of leaves from 80 species of plants during 24 h laboratory experiment (x ± SD, n = 7)

Family	Species	Part of plant	Mortality of nematode larvae in 3.0% plant solution, %	Mortality of nematode larvae in control, %	Reliability of impact of 3.0% plant solution compared with the control
Amaryllidoideae	*Narcissus poeticus* L.	leaves	20.3 ± 7.5	17.5 ± 8.1	–
Apiaceae	*Daucus carota* L.	leaves	80.4 ± 5.8	19.0 ± 6.5	***
Apiaceae	*Eryngium campestre* L.	leaves	95.6 ± 4.7	15.7 ± 4.0	***
Apiaceae	*Falcaria vulgaris* Bernh.	leaves	95.2 ± 6.4	22.9 ± 15.8	***
Apocynaceae	*Asclepias syriaca* L.	leaves	34.3 ± 7.4	22.5 ± 6.5	–
Aristolochiaceae	*Aristolochia clematitis* L.	leaves	57.4 ± 10.6	17.5 ± 8.1	**
Asparagaceae	*Asparagus officinalis* L.	shoots	49.0 ± 6.2	17.5 ± 8.1	*
Asteraceae	*Achillea millefolium* L.	inflorescences	99.2 ± 1.1	15.4 ± 4.4	***
Asteraceae	*Anthemis subtinctoria* Dobrocz.	inflorescences	41.7 ± 7.4	19.6 ± 8.8	*
Asteraceae	*Artemisia vulgaris*L.	shoots	62.5 ± 11.2	22.5 ± 8.6	**
Asteraceae	*Calendula officinalis* L.	inflorescences	54.7 ± 7.4	19.6 ± 8.8	**
Asteraceae	*Carduus acanthoides* L.	leaves	88.4 ± 7.9	27.8 ± 19.1	***
Asteraceae	*Centaurea scabiosa* L.	leaves	50.7 ± 9.1	22.5 ± 6.5	*
Asteraceae	*Cichorium intybus* L.	shoots	47.8 ± 9.3	22.1 ± 10.4	*
Asteraceae	*Cyclachaena xanthiifolia* (Nutt.) Fresen.	leaves	20.7 ± 7.0	11.8 ± 6.2	–
Asteraceae	*Erigeron annuus* (L.) Desf.	shoots	95.8 ± 5.9	11.0 ± 7.4	***
Asteraceae	*Galatella villosa* (L.) Rchb. f.	shoots	65.2 ± 8.9	14.3 ± 6.7	**
Asteraceae	*Grindelia squarrosa* (Pursh.) Dunal	shoots	100.0 ± 0.0	33.0 ± 6.0	***
Asteraceae	*Hieracium umbellatum* L.	shoots	67.3 ± 8.5	33.0 ± 8.5	***
Asteraceae	*Lactuca serriola* L.	shoots	59.8 ± 10.4	22.5 ± 8.6	**
Asteraceae	*Tragopogon orientalis* L.	leaves	97.5 ± 1.6	20.4 ± 8.1	***
Asteraceae	*T. podolicus* (DC.) S. A. Nikitin	leaves	94.2 ± 7.1	20.4 ± 8.1	***
Boraginaceae	*Echium vulgare* L.	shoots	26.1 ± 8.1	11.8 ± 6.2	–
Boraginaceae	*Nonea pulla* (L.) DC.	shoots	50.3 ± 9.0	22.1 ± 10.4	*
Brassicaceae	*Descurainia sophia* (L.) Webb ex Prantl	shoots	44.3 ± 8.4	17.5 ± 8.1	*
Campanulaceae	*Campanula sibirica* L.	shoots	62.8 ± 8.7	17.5 ± 8.1	**
Caryophyllaceae	*Gypsophila paniculata* L.	shoots	55.9 ± 9.1	14.3 ± 6.7	**
Caryophyllaceae	*Silene dichotoma* Ehrh.	shoots	66.7 ± 10.9	22.5 ± 8.6	**
Caryophyllaceae	*S. sibirica* (L.) Pers.	shoots	67.4 ± 11.4	22.1 ± 10.4	**
Convolvulaceae	*Convolvulus arvensis* L.	shoots	57.4 ± 8.0.	11.8 ± 6.2	*
Cornaceae	*Cornus sanguinea* L.	leaves	22.6 ± 7.6	17.5 ± 8.1	–
Dipsacaceae	*Knautia arvensis* (L.) J. M. Coult.	shoots	27.9 ± 6.2	22.1 ± 10.4	–
Ericaceae	*Vaccinium vitis-idaea* L.	shoots	48.5 ± 7.3	17.5 ± 8.1	*
Euphorbiaceae	*Euphorbia stepposa* Zoz ex Prokh.	shoots	46.3 ± 9.2	14.3 ± 6.7	*
Fabaceae	*Astragalus cicer* L.	shoots	70.7 ± 10.7	22.5 ± 6.5	**
Fabaceae	*Chamaecytisus austriacus* (L.) Link	shoots	68.5 ± 8.7	19.6 ± 8.8	**
Fabaceae	*Genista tinctoria* L.	shoots	98.5 ± 1.0	33.0 ± 6.0	***
Fabaceae	*Lathyrus tuberosus* L.	shoots	49.2 ± 8.1	14.3 ± 6.7	*
Fabaceae	*Medicago falcata* L.	shoots	74.0 ± 12.4	14.3 ± 6.7	**
Fabaceae	*M. lupulina* L.	shoots	31.9 ± 6.0	11.8 ± 6.2	–
Fabaceae	*M. sativa* L.	shoots	94.0 ± 5.5	19.0 ± 6.5	***
Fabaceae	*Melilotus albus* Medik.	shoots	57.4 ± 6.9	22.5 ± 6.5	**
Fagaceae	*Quercus robur* L.	leaves	21.9 ± 7.0	11.8 ± 6.2	–
Fagaceae	*Q. robur* L.	bark	17.5 ± 9.3	14.3 ± 6.7	–
Hypericaceae	*Hypericum perforatum* L.	shoots	62.7 ± 10.7	22.1 ± 10.4	**
Lamiaceae	*Ballota nigra* L.	shoots	31.9 ± 6.5	14.3 ± 6.7	–
Lamiaceae	*Mentha × piperita* L.	shoots	98.6 ± 2.9	19.0 ± 6.5	***
Lamiaceae	*Phlomis pungens* Willd.	shoots	71.3 ± 4.8	22.5 ± 8.6	***
Lamiaceae	*Salvia nutans* L.	shoots	96.3 ± 3.1	27.8 ± 19.1	***
Lamiaceae	*S. tesquicola* Klokov & Pobed.	shoots	59.4 ± 10.6	14.3 ± 6.7	**
Lamiaceae	*Stachys recta* L.	shoots	96.7 ± 3.8	27.8 ± 19.1	***
Lamiaceae	*Teucrium polium* L.	shoots	96.4 ± 2.7	33.0 ± 6.0	***
Lamiaceae	*Thymus marschallianus* Willd.	shoots	14.3 ± 4.9	12.0 ± 6.2	–
Linaceae	*Linum hirsutum* L.	shoots	60.7 ± 9.2	11.8 ± 6.2	**
Malvaceae	*Lavatera thuringiaca* L.	leaves	97.4 ± 3.2	15.7 ± 4.0	***
Oleaceae	*Syringia vulgaris* L.	leaves	39.0 ± 6.3	17.5 ± 8.1	*
Onagraceae	*Chamaenerion angustifolium* (L.) Scop.	leaves	20.3 ± 6.2	19.6 ± 8.8	–
Onagraceae	*Epilobium palustre* L.	shoots	22.4 ± 6.6	17.5 ± 8.1	–
Poaceae	*Aegilops cylindrica* Host	shoots	56.2 ± 6.8	22.5 ± 6.5	**
Poaceae	*Bromopsis inermis* (Leyss.) Holub	shoots	86.4 ± 8.7	20.4 ± 8.1	***
Poaceae	*Bromus squarrosus* L.	shoots	50.0 ±11.3	22.5 ± 8.6	*
Poaceae	*Calamagrostis epigejos* (L.) Roth	shoots	53.2 ± 7.6	22.5 ± 8.6	*
Poaceae	*Triticum aestivum* L.	shoots	51.1 ± 9.4	19.6 ± 8.8	*
Poaceae	*Zea mays* L.	stamen filaments	52.4 ± 7.4	19.6 ± 8.8	**
Polygonaceae	*Polygonum aviculare* L.	shoots	60.0. ± 8.6	14.3 ± 6.7	**

Table 1 continued...

Family	Species	Part of plant	Mortality of nematode larvae in 3.0% plant solution, %	Mortality of nematode larvae in control, %	Reliability of impact of 3.0% plant solution compared with the control
Ranunculaceae	*Nigella arvensis* L.	shoots	51.2 ± 7.8	11.8 ± 6.2	**
Ranunculaceae	*Thalictrum minus* L.	leaves	97.8 ± 2.8	28.7 ± 4.8	***
Resedaceae	*Reseda lutea* L.	shoots	52.2 ± 5.8	19.6 ± 8.8	*
Rosaceae	*Agrimonia eupatoria* L.	leaves	44.5 ± 10.4	22.1 ± 10.4	–
Rosaceae	*Cerasus fruticosa* Pall.	leaves	96.7 ± 2.2	21.7 ± 5.3	***
Rosaceae	*Fragaria vesca* L.	leaves	45.1 ± 12.4	19.6 ± 8.8	*
Rosaceae	*Malus sylvestris* Mill.	leaves	97.4 ± 1.2	33.0 ± 8.5	***
Rosaceae	*Potentilla argentea* L.	shoots	22.5 ± 8.0	17.5 ± 8.1	–
Rosaceae	*Rubus caesius* L.	shoots	16.7 ± 4.9	14.3 ± 6.7	–
Sapindaceae	*Acer tataricum* L.	shoots	26.2 ± 5.0	22.5 ± 8.6	–
Solanaceae	*Licium barbarum* L.	shoots	80.8 ± 5.3	14.3 ± 6.7	***
Typhaceae	*Typha latifolia* L.	leaves	48.8 ± 11.2	22.5 ± 8.6	*
Ulmaceae	*Ulmus laevis* Pall.	shoots	92.5 ± 4.5	28.7 ± 4.8	***
Ulmaceae	*U. minor* Mill.	shoots	98.8 ± 2.0	21.7 ± 5.3	***
Urticaceae	*Urtica dioica* L.	shoots	91.7 ± 5.3	19.0 ± 6.5	***
Violaceae	*Viola ambigua* Waldst. & Kit.	leaves	35.2 ± 7.2	22.1 ± 10.4	–

Reliability of differences in mortality of larvae in the control and plant solution was calculated using ANOVA: * – P< 0.05, ** – P< 0.01, *** – P< 0.001.

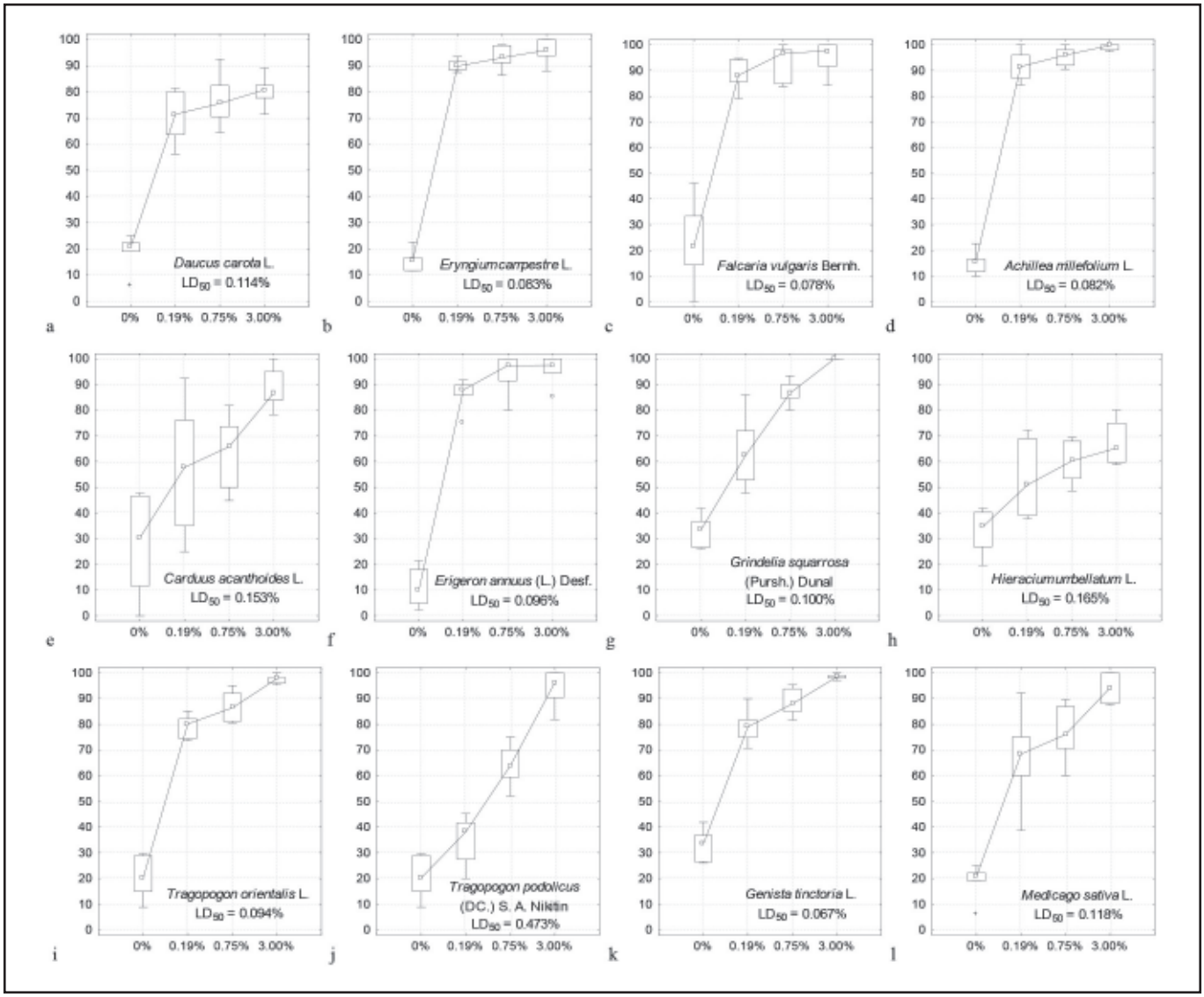

Figure 1. Influence of aqueous tinctures of Apiaceae (a–c), Asteraceae (d–j) and Fabaceae (k, l) on mortality of rhabditiform larvae (L_{1-2}) of *S. papillosus* during 24 h experiment (n = 7).

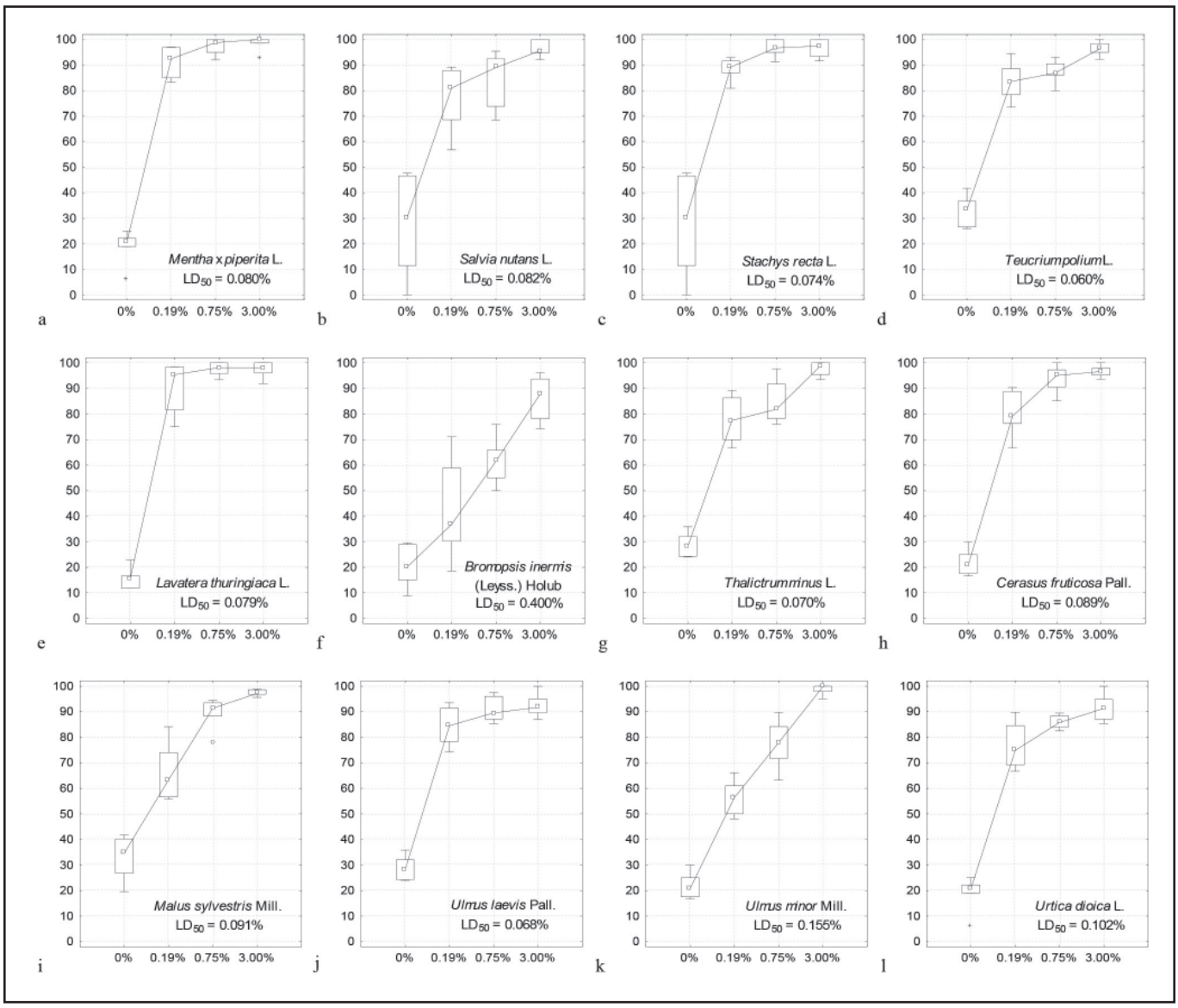

Figure 2. Influence of aqueous tinctures of plants of families Lamiaceae (a–d), Malvaceae (e), Poaceae (f), Ranunculaceae (g), Rosaceae (h, i), Ulmaceae (j, k) and Urticaceae (l) on mortality of rhabditiform larvae ($L_{1–2}$) of *S. papillosus* during 24 h experiment (n = 7).

(Urticaceae) in the concentration of 0.19% affected less than 80% of the studied stages of larvae of *S. papillosus* (Figure 2l).

Highest parameters of LC_{50} (0.060–0.069%) regarding $L_{1–2}$ *S. papillosus* belonged to *T. polium*, *A. millefolium*, *G. tinctoria* and *U. laevis*. Lower parameters (LC_{50} = 0.070–0.079%) were recorded for *T. minus*, *S. recta*, *F. vulgaris*, *L. thuringiaca*. This parameter was no lower than 0.08% for *M. × piperita*, *A. millefolium*, *S. nutans*, *E. campestre* and *C. fruticosa*. Aqueous tinctures of *M. sylvestris*, *T. orientalis*, *E. annuus*, *G. squarrosa*, *U. dioica*, *D. carota*, *M. sativa*, *C. acanthoides*, *U. minor* and *H. umbellatum* exhibited less notable nematicidal properties against $L_{1–2}$ *S. papillosus* (LC_{50} = 0.090–0.165%). Parameter LC_{50} for *B. inermis* and *T. podolicus* was even lower – 0.40% and 0.47%, respectively (Figure 1, 2).

DISCUSSION

In the present study, the plants from the Samarsky Bor Regional Landscape Park (Ukraine) were studied for nematicidal properties against $L_{1–2}$ of *S. papillosus* in *in vitro* experiments. The *in vitro* evaluation showed that 20 species of plants cause death to $L_{1–2}$ *S. papillosus*. Among them, 13 species (*E. campestre*, *F. vulgaris*, *A. millefolium*, *E. annuus*, *T. orientalis*, *G. tinctoria*, *M. × piperita*, *S. nutans*, *S. recta*, *T. polium*, *L. thuringiaca*, *C. fruticosa*, *U. laevis*) were effective against $L_{1–2}$ even in the lowest concentration of aqueous tinctures (0.19%).

Surveys on anthelmintic properties of plants against species of the *Strongyloides* genus have also been conducted by other authors. Anthelmintic activity of *Piper retrofractum* Vahl has been studied in *in vitro* conditions against L_3 of S. *stercoralis* (Bavay, 1876) (Sangkhantree *et al.*, 2018; Riyong *et al.*, 2020). Boonmars *et al*. (2005) reported antiparasitic activity of *Cardiospermum halicacabum* L., and El-Sherbini & Osman (2013) – *Mangifera indica* L. against larvae of the same stage of the development. Ismail *et al.* (2016) determined anthelmintic properties of *Lawsonia inermis* L. against larvae of *Strongyloides* spp. Moraes *et al*. (2017) and Cabral *et al.* (2019) studied the impact of some constituents of *Carica papaya* L. on eggs and larvae of *Strongyloides venezuelensis* (Brumpt, 1934). Extract of *Siparuna guianensis* Aublet also exhibited high anthelmintic activity (Carvalho *et al.*, 2019)

towards eggs and larvae of *S. venezuelensis* Brumpt, 1934. Ethanol extract from *Spondias mombin* L. and its aqueous fraction (Bastos *et al.*, 2017) also demonstrated 100% *in vitro* efficiency against parasitic females of *S. venezuelensis*. Similar experiments (Rehder *et al.*, 2014) were also performed using *Labramia bojeri* A. DC.

The impact of other plants on the nematode *S. papillosus* has been reported earlier (Boyko & Brygadyrenko, 2016, 2019; Boyko *et al.*, 2020). Among the species of plants studied earlier, significant impact on L_{1-2} *S. papillosus* was exerted by *Salvia sclarea* L.*, Matricaria chamomilla* L., *Petroselinum crispum* (Mill.), *Taraxacum officinale* F. H. Wigg., *Sanguisorba officinalis* L., *Ambrosia artemisiifolia* L., *Arctiumminus* (Hill) Bernh., *Lotus ucrainicus* Klok., *Juniperus communis* L., *J. sabina* L., *Thuja occidentalis* L., *Aristolochia manshuriensis* Kom., *Celastrus scandens* L., *Colchicum autumnale* L., *Laburnum anagyroides* Medik., *Wisteria sinensis* (Sims) DC., *Quercus petraea* subsp. *iberica* (Steven ex M. Bieb.) Krassiln., *Ginkgo biloba* L., *Magnolia kobus* DC., *Ailanthus altissima* (Mill.) Swingle.

In this study, the shoots of *S. nutans* had influence similar to inflorescences of *S. sclarea*. Exposure to aqueous tinctures led to death of more than 80% of L_{1-2} *S. papillosus* in all the tested concentrations (3.00%, 0.75%, and 0.19%). Differences between the results were seen for the effect of *S. tesquicola*. Aqueous tincture of its shoots in 3% concentration caused death to about 60% of larvae. Significant differences were also seen for plants of *Quercus* genus. Tinctures of *Q. robur* had no effect on vitality of larvae, unlike *Q. petraea* subsp. *iberica*, in 3% aqueous tincture of which the vitality of larvae did not exceed 6%. Interesting results were observed for the use of *M. falcata*, *M. lupulina*, and *M. sativa*. The strongest effect on L_{1-2} *S. papillosus* was shown by aqueous tincture of above-ground part of plants of *M. sativa*. Such differences perhaps may be related to species peculiarities of chemical composition of plants of the *Medicago* genus.

Experiments in *in vitro* conditions regarding the effect of alcohol extract of *M. piperita* on the development of eggs and nematode larvae (*Haemonchus contortus* (Rudolphi, 1803) were performed by Carvalho *et al*. (2012): LC_{50} of mint extracts accounted for 37 mg/L regarding the development of eggs, and 18 mg/L regarding larvae. In our surveys, the value of LC_{50} for *M. piperita* was 80 mg/L, indicating higher efficiency of alcohol extract compared with aqueous tincture.

The conditions of maintenance and feeding of agricultural animals are among the main factors of their productivity (Zazharska *et al.*, 2018). Currently, many studies focus on the intensity of helminthic diseases in the conditions of pasture grazing of animals. Some pasture plants have anthelmintic properties. Marley *et al*. (2005) demonstrated that lambs grazing on lucerne had fewer adult nematodes of *Teladorsagia circumcincta* (Stadelman, 1894) than lambs grazing on ryegrass or red clover. Our *in vitro* results also indicate the anthelmintic properties of *M. sativa*. Over 90% of larvae died in 3% aqueous solution. Niezen *et al*. (2002) demonstrated that in feces of lambs which grazed *M. sativa* in the pasture in summer and autumn, the amount of eggs of *Trichostrongylus colubriformis* was insignificantly reduced compared to the control. Athanasiadou and Houdijk (2010) describe an experiment with grazing animals on bioactive forages. The best results were obtained with *C. intybus*. The studies regarding the *in vitro* effect of aqueous tincture of *C. intybus* on L_{1-2} of *S. papillosus* revealed no nematicidal properties in this plant. Over 50% of larvae remained vital during 24 h in different concentrations. Perhaps, the difference of our results is associated with performing the experiment *in vitro*, and not *in vivo*.

CONCLUSION

Study of anthelmintic properties of pasture plants are of great importance as an alternative treatment of helminthiases of animals. Out of 80 species of plants used in the study, only 20 species of plants exerted nematicidal activity towards L_{1-2} *S. papillosus*. Among them, 13 species (*E. campestre*, *F. vulgaris*, *A. millefolium*, *E. annuus*, *T. orientalis*, *G. tinctoria*, *M.* × *piperita*, *S. nutans*, *S. recta*, *T. polium*, *L. thuringiaca*, *C. fruticosa*, *U. laevis*) effectively affected L_{1-2} even in lowest (0.19%) concentration of the tested aqueous tinctures. Lowest parameters of LC_{50} (0.060–0.069%) regarding larvae of *S. papillosus* were recorded for *T. polium*, *A. millefolium*, *G. tinctoria* and *U. laevis*. These results would be interesting for future *in vivo* experiments in pasture conditions so as to decrease the use of synthetic anthelmintic preparations.

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Competing Financial Interests
The authors declare no competing interests.

Conflict of Interest
The authors declares that they have no conflict of interests.

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