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BIOLOGICAL ACTIVITY OF EXTRACTS FROM SOME SPECIES OF CONIFEROUS PLANTS

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The development and use of biological preparations based on plant secondary metabolites is an important trend in modern organic farming. Among secondary metabolites, a significant place is belong to substances of the class of terpenoids (isoprenoids), which possess a wide spectrum of biological activity and practically no toxicity to warm-blooded animals and humans. One of the main sources of terpenoids is conifers, such as *Juniperus sabina* L. (Cupressaceae) and *Pinus sylvestris* L. (Pinaceae), which in quantitative content and qualitative composition are vastly superior to other widely distributed species in the plant world. Thus, the secondary metabolites of savin juniper and scots pines advantageous as a raw material for the production of various biological preparations for organic agriculture. The aim of this study was to determine the biological activity of extracts and essential oils from species of *Juniperus sabina* and *Pinus sylvestris*, growing in the Republic of Moldova (RM) and Slovak Republic, Nitra (SN) and Pianiny mountains (PM). The antioxidant activity of ethanolic extracts of Coniferous was determined by two procedures with appreciation of their radical scavenging capacities against DPPH and peroxyl free radicals and was evaluated in equivalent of standard substances (Trolox and gallic acid) and by indexes IC_{50}. According to the determined indexes the antioxidant activity of tested extracts from Coniferous plants was qualified in followed order: *J. sabina* SN > *J. sabina* PM > *J. sabina* RM> *P. sylvestris* RM ≥ *P. sylvestris* SN. Moreover, it was established that both ethanolic extracts and essential oil *J. sabina* possessed high efficiency against *Leptinotarsa decemlineata* Say and *Galleria mellonella* L. Mortality of imago and larvae consisted in average 6.7–53.3 and 66.7–100% respectively, ovicidal activity was 100%, and antifeedant effect persisted at the level of 1–3 points. The treatment of feed and insects with the essential oils of *P. sylvestris* needles resulted in the death of only 6.7–13.3% of the wax moth larvae.

Keywords: Coniferous plant, extract, essential oil, antioxidant activity, biopesticidal property

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Introduction

Plants play a significant role in human life. They are a source of food, serve as raw materials for the construction and manufacture of various products, applied in almost all areas of human activity, including medicine, perfume, light and coatings industries, etc. Generally, the phytochemical constituents of plants break up into two categories based on their role in basic metabolic processes, namely primary and secondary metabolites. Primary plant metabolites are involved in basic life functions; therefore, they are more or less similar in all living cells. These include amino and nucleic acids, proteins, lipids, carbohydrates, enzymes, vitamins, and some organic acids. The role of secondary plant metabolites (SPMEs) in growth, photosynthesis, reproduction, and development of plants has not been found yet. At the same time these substances play an important role in plant survival in the environment and serve to establish ecological relationships between plants and other organisms. In the course of their vital activity, higher plants accumulate a big number of SPMEs (estimated 200,000), belong to the wide variety of chemical classes of compounds: alkaloids, terpenoids, saponins, phenolic compounds (flavonoids, tannins) and their glycosides (Crozier et al., 2006). Due to chemical structure, the SPMEs can exhibit a large spectrum of biological activity (growth regulator, immunomodulator, antioxidant, antimicrobial, pesticide, etc). The main advantages of preparation based on secondary plant metabolites are their high activity at low concentrations, specificity, harmlessness for the environment, low toxicity for warm-blooded animals, humans, and many species of beneficial insects, including pollinators. In this context, the development and use of biological preparations based on plant secondary metabolites is an important trend in modern organic farming.

Savin juniper or savin (*Juniperus sabina* L., Cupressaceae) is one of the most widespread conifer species in the world, native to the mountains of central and southern Europe, western and central Asia, as well as Northern Africa (Algeria). It grows in a wide variety of ecological conditions: on the sand, chalk deposits, rocks, foothills and high in the mountains, rising to 3,300 m, occurring on southern stony and fine-earthed northern slopes. Needles of *J. sabina* contain bitter glycoside pinopicrin, gallic acid, tannins, flavonoids, resin, wax, vitamin C, organic acids and more. In addition, savin juniper contains essential oil (up to 2.5–4.8%), which has a specific odor and consists of alcohol sabinol and various terpenic compounds (Ayoshina and Velichko, 2004; Myrzagaliyeva and Medeubaeva, 2014; Novikov et al., 2014). Such a variety of chemical compounds, as well as the availability of extraction of biologically active substances from various plant organs, has led to the fact that the savin is widely used in medicine and homeopathy, perfumery, in microscopic technique (immersion oil), for the production of various alcohol drinks, as well as dyes, lacquers, joinery, bath besom, etc. (Lagoni, 2002; Aleksandrova and Aleksandrov, 2005; Farjon, 2005, 2013; Nosov, 2007; Semenyutina et al., 2014).

Scots pine (*Pinus sylvestris* L., Pinaceae) is a long-living, coniferous tree that is the most widely distributed pine, native to Eurasia. It is readily identified by its combination of fairly short, blue-green needles and orange-red bark. The needles are 3–5 cm long and occur in fascicles of two. The cones are pointed ovoid in shape and 3–7 cm long. The threes normally grow up to 25 m in height and in exceptional cases up till 40 m. Scots pine is, especially in the north of Europe, an economically important species. The wood is strong and easy to work with, making it excellent for general constructions, furniture, and the pulp and paper industry. Pine, as well as juniper, is also used for stabilizing sandy soils (Gardner, 2013; Daugavietis and Spalvis, 2014). Scots pine is an important timber tree. Earlier it uses as mining props and for interior construction, for street paving blocks. At the moment most of the production goes to the paper industry. Other uses of scots pine wood are railway sleepers, fencing, crates, pallets, boxes, laminated wood, particleboard, fibreboard, and various wood-based materials (Gardner, 2013; Daugavietis and Spalvis, 2014).

The secondary metabolites of savin juniper and pines have long attracted the attention of researchers as a raw material for the production of various biological preparations for organic agriculture. Earlier, it was shown that the *J. sabina* and *P. sylvestris* possessed potential biological activity (Elisovetcaia et al., 2017, 2018a, b; Gladei et al., 2018; Elisovetcaia and Brindza, 2018). The purpose of this research was to establish the antioxidant and biopesticidal activity of extracts and essential oils from species of *Juniperus sabina* and *Pinus sylvestris*, growing in the Republic of Moldova and Slovak Republic.

Material and methodology

Scientific researches were carried out in the laboratory conditions during 2017–2019 years, in the Laboratories of Bioreglators Natural and Integrated Protection, Institute of Genetics, Physiology and Plant Protection (IGPPP), Chisinau, Republic of Moldova and in the Institute of Biodiversity Conservation and Biosafety, Department of Genetics and Plant Breeding (FAaFR), Slovak University of Agriculture in Nitra.

Plant materials

The needles (twigs) of *Juniperus sabina* were collected in spring and autumn 2017–2018, and *Pinus sylvestris* in autumn 2018 and in winters 2018–2019 in the different zones from Slovak Republic (Nitra and Tatras Mountains, Pianin region) and Republic of Moldova, dried and crushed used the laboratory mill Type MRP-1 (asynchronous motor, 'Agropribor', Russia) and rotary chopper PULVERISETTE, powdered to uniform size of particles passing through a sieve with apertures 2.0 mm in diameter. The plant materials were collected by trimming 30–40 cm of regrown basal branches. The identification of the *J. sabina* and *P. sylvestris* plants was carried out by consultation with the specialists from the Botanical Garden (Institute) and the Forest Research and Management Institute – ICAS, Chisinau, as well as by morphometric analysis of juniper and pine needles. The following abbreviation was accepted depending on the region where the plant materials were collected: Slovakia, Nitra – SN; Slovakia, Tatras Mountains, Pianin region – SM and Republic of Moldova – RM.

Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

The content of moisture and dry substances

The content of moisture and dry substances were determined both for fresh and dried herbal raw materials, as well as for extracts according to the standard methods using Moisture analyzer MAX series, RADWAG 26 – 600 Radom (Markova et al., 2003).

Preparation of ethanolic extracts

The dry, crushed and powdered plants raw materials from *Juniperus sabina* and *Pinus sylvestris* were extracted with 96% ethyl alcohol in the ratio plant materials: solvent – 1 : 5. The mixture was shacked for 4 hours on a laboratory shaker, then infused during 72 hours, followed by evaporation of the solvent. The precipitation was diluted with 96% ethanol and the extract, containing in 100 ml 20 g of dry substances, was obtained.

Essential oil

Essential oil extraction was carried out by the hydrodistillation Ginsberg method (Rassem et al., 2016; Timasheva et al., 2016). A samples (200 g) fresh or dry plant raw materials were placed in a 2 liter flat bottomed flask, 1,600 ml of water was added and boiled. The duration of this procedure was 4.0 hours. Essential oil was evaporated from plant raw materials by heating a mixture of water and plant materials at atmospheric pressure, followed by the liquefaction of the vapors in a condenser (reflux) and collection in receiving vessel Ginsberg. The yield of essential oil was expressed as a mass-volume percent of absolute dry weight of the plant raw materials.

Determination of peroxyl radical scavenging activity (PRSA)

Determination of PRSA of extracts is based on the degree of inhibition of potassium iodide oxidation by antioxidants that scavenge peroxyl radicals, generated from thermal degradation of 2.2‘-azobis(2-amidinopropane)-dihydrochloride (Ivanova, 2016). Iodine amount released during oxidation was determined by potentiometric titration using the automatic titrator “Titroline easy” (Germany) with steps of 0.01 ml. PRSA of the tested sample was expressed as the inhibition ratio for iodine release in comparison with control sample (without antioxidant). The PRSA of standard antioxidants and plant extracts progressed in a dose-dependent manner. The linear relationship between PRSA and concentration of samples containing antioxidants had high correlation coefficients (r^2 = 0.848–0.997). The 50% inhibitory concentration IC (50) of each extract was calculated from curves of dose-dependent PRSA. The doses of standard antioxidants that scavenged 50% of free radicals were 35.37 ±3.36 µM for gallic acid and 138.71 ±22.33 µM for Trolox. PRSA of extracts was expressed in gallic acid (GAE) equivalent.

DPPH radical scavenging activity

DPPH radical scavenging activity of extracts was measured using 2.2-diphenyl-1-picrylhydrazyl (DPPH) by procedure widely reported (Clarke et al., 2013). The tested extracts of different concentrations (from 0.1 to 1%) were prepared. The sample (0.1 mL) was mixed with 3.9 mL of DPPH solution (0.025 g DPPH in 100 mL methanol). The absorbance of the reaction mixture was determined after 10 min. in darkness using the Visible Spectrophotometer GENESYS™ 20 (4001-000 UV/Vis, ThermoFisher Scientific) at 515 nm. The inhibition concentration of 50% free radicals (IC_{50}) was calculated from the curve of activity-dose dependence in limits of extract concentration from 25 to 500 µg/ml. The Trolox (6-hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid, 0–210 µg/L, R^2 = 0.998) serve as standard antioxidant substances and radical scavenging activity of tested extracts were expressed in mg Trolox equivalents per g DW.

Determination of ovicidal, insecticidal, antifeedant, repellent and deterrent activities in the laboratory conditions

Determination of biopesticidal activity of alcohol extracts and essential oil were carry out on the egg-laying, imago and 2–3 instars larvae of Colorado Potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) and on the 2–3 instars larvae wax moth *Galleria mellonella* L. (Linnaeus, 1758) (Lepidoptera: Pyralidae) according to classic method which was modified us (Elisovetcaia, 2010). The biological material of *L. decemlineata* was obtained from a natural population of insects, but *G. mellonella* was reared in the laboratory conditions using an artificial nutrient medium.

Laboratory testing of the extracts against *L. decemlineata* L. was carried out in a climate cell with adjustable conditions at set temperatures of +22–24 °C, air humidity of 70–80% and 16-hours photoperiod. Young leaves of potatoes with standard sizes have served as a substrate for feeding *L. decemlineata*. Leaves were treated using a method of immersing into the extracts' and oils' solutions, kept in laboratory exhaust hood for 1 hour until full evaporation of the solvent and after which they were placed into Petri dishes with insects. The control was a variant of leaves treatment with a water-ethanolic solution of corresponding concentration.

Laboratory testing of the extracts and oils from *J. sabina* and *P. sylvestris* against *G. mellonella* was carried out in a climate cell Versatile Environmental Test Chamber SANYO with adjustable conditions at set temperatures of +28.0–0.2 °C, air humidity of 70% and 16-hours photoperiod. For laboratory experiments, Petri dishes were used. An artificial nutrient medium (ANM) has served as a feed for *G. mellonella* with the addition of the extracts and oil. Were 11 variants: first to third three controls – treatment of the feed (200 µkl/2.0 g feed) and dorsal field of the insects (5 µkl) with 0.5% ethanolic solution and without treatment as well as fourth to eleventh – treatment of the feed (200 µkl/2.0 g feed) and treatment of the dorsal field of the insects (5 µkl) with ethanol extracts (0.1%) and essential oil from *J. sabina* and *P. sylvestris*. The feed was treated by essential oils and the plant extracts using a micropipette and thoroughly mixed kept outdoors for an hour and then placed into Petri dishes with insects. We observed the movement of larvae during the first hour after the accommodation and then daily monitored the behavior of the larvae. Also, the feed was periodically weighed (on 3, 7, 14 and 18 days), up to the beginning of pupation of the larvae.

Insecticidal activity

The insecticidal activity (efficiency) was determined using the number of dead insects for three days (for *L. decemlineata*) and two weeks (for *G. mellonella*) in comparison with the control according to a classic method which was modified by us (Elisovetcaia, 2010).

Antifeedant activity

The antifeedant activity of the extracts and oils under laboratory conditions against Colorado potato beetle was established on the third day after the beginning of the experiment according to a standard scale in points (Elisovetcaia, 2010); against larvae *G. mellonella* was noted by the amount of feed eaten by one larva in three days compared to the control.

Repellent effect

Repellent effect was recorded on the basis of the behavior of the larvae and imago *L. decemlineata* and of the larvae *G. mellonella* after placing them in Petri dishes with the treated leaves or nutrient medium.

Effect on pupal and imago phases (deterrent activity)

The effect of alcohol extracts and essential oils from plants of *J. sabina* and *P. sylvestris* was evaluated when larvae fed with treatment meal, as well as with topical dorsal application. Observations on insects were carried out periodically for a month. It was noted the beginning of pupation and the emergence of the imago, as well as the number of eggs laid.

Ovicidal activity

The extracts (0.1–20.0%) and oils (0.1%) from *J. sabina* were tested on ovicidal activity using egg clutches of *L. decemlineata*, collected from untreated potato plants. Each variant consisted of three replications, three-egg clutches each. Leaves with egg clutches were treated using a method of immersing into the extract for several seconds and then kept in an exhaust hood for 1 hour until full evaporation of the solvent. Then they were placed into double dishes. Leaves with egg clutches treated with water-ethanolic solution of corresponding concentration served as a control. The ovicidal activity was determined on the 10th day according to classic method which was modified us (Elisovetcaia, 2010).

Statistic analysis

Statistical processing of obtained data was carried out according to the one-way ANOVA test and Microsoft Excel software.

Results and discussion

It was found that the moisture of the needles of juniper savin and scots pine varies depending on the place of growth and on the collection period (Table 1). On average, the annual content of moisture of needles *J. sabina* fluctuated from 45.15 ±1.30 (February) to 59.08 ±2.45% (May). The needles of *J. sabina* growing in Nitra accumulated the maximum amount of moisture, the smallest – in Moldova. At the same time, the annual average of needles *P. sylvestris* moisture fluctuated from 47.10 ±2.15 (March) to 58.60 ±2.30% (September). The plants of *P. sylvestris* growing in Moldova accumulated the maximum amount of moisture, the smallest – in Nitra. A comparative analysis of the annual average of moisture content of raw materials showed that there is an insignificant difference between the plant samples from different collection regions, for *J. sabina* and *P. sylvestris*. However, it should be noted that during the season (spring, autumn, winter) the moisture content of the raw material varies significantly in all regions for both Coniferous plants (Table 1).

Table 1 Annual average of moisture content in needles of *Juniperus sabina* L. and *Pinus sylvestris* L. from different places of growth

Variant	Average of moisture content depending on the time of collection (%)			Annual average of plant raw materials moisture (%)	Difference between variants	Least significant difference
	spring	autumn	winter			
Juniperus **sabina, Moldova, Chisinau (RM)**	47.80 ±1.75	46.20 ±1.50	45.15 ±1.30	46.38 ±0.92	–	*$LSD_{0.05}$ =8.27, p >0.05
Juniperus **sabina, Slovakia, Pianiny, Tatras Mountains (SM)**	51.03 ±2.12	50.17 ±2.45	49.30 ±1.90	50.17 ±2.45	-3.79	
Juniperus **sabina, Slovakia, Nitra (SN)**	59.08 ±2.45	52.50 ±2.15	47.10 ±2.45	52.89 ±5.99	-6.51	
	**$LSD_{0.05}$ = 0.79, p ≤0.05					
***Pinus sylvestris*, Moldova, Chisinau (RM)**	50.80 ±3.25	58.60 ±2.30	50.36 ±1.85	53.25 ±4.84	-	*$LSD_{0.05}$ = 12.35, p >0.05
***Pinus sylvestris*, Slovakia, Nitra (SN)**	48.10 ±1.88	56.80 ±2.78	49.07 ±2.01	51.32 ±4.76	1.93	
	**$LSD_{0.05}$ = 0.91, p ≤0.05					

Notes: RM – Republic of Moldova; SN – Slovakia, Nitra; SM – Slovakia, Pianiny; Tatras mountains region

The analysis of the antioxidant capacities of extracts *J. sabina* and *P. sylvestris*, growing in different zones of Slovakia and in R. of Moldova is shown in Table 2. The extracts of *P. sylvestris* from Slovakia and Moldova possessed the highest IC_{50} in comparison with the extracts of *J. sabina*. The extract of *J. sabina* from Nitra had significantly lower IC_{50} than the extracts from Pianiny Mountains and Moldova. The lowest IC_{50} represents the highest potency of extracts to scavenge of DPPH free radicals. It was noted, that the DPPH radical scavenging activity of ethanolic extracts of J sabina depended on places of growth. At the same time, it was revealed that the antioxidant activity of *P. sylvestris* extracts growing in different regions does not significantly differ from each other ($LSD_{0.05}$ = 5.3; p ≤0.05). The ethanolic extracts of *J. sabina* from Nitra showed the highest antioxidant activity against both DPPH and peroxyl free radicals. The antioxidant activity of extracts of *J. sabina* from Moldova was significantly lower than other samples of *J. sabina* (Table 2). Despite the fact that extracts from scots pine showed an activity of 2.6–4.4 times lower than that of savin juniper, their antioxidant activity can also be considered high. Thus, all tested samples conform to diminution of antioxidant activity that was presented by followed order: *J. sabina* SN > *J. sabina* PM > *J. sabina* RM> *P. sylvestris* RM ≥ *P. sylvestris* SN. It should be noted that to scavenge the same amount of DPPH free radicals the extracts were used in concentrations of 14–16 times more than for peroxyl radical scavenging. The results (Table 2) obtained by both procedures for determinations of antioxidant activities have close correlation, Pearson coefficient of correlation equal to 0.98–0.99. These results coincided well with our previous studies (Gladei et al., 2018; Elisovetcaia et al., 2018a).

Table 2 Radical scavenging activity of extracts from needles of *Juniperus sabina* L. and *Pinus sylvestris* L., various places of growing

Sample	DPPH antioxidant activity		Peroxyl radical scavenging activity		Gallic acid equivalent (GAE) (μMol/g DW)
	IC_{50} (mg/ml)	Trolox equivalent (μg/g DW)	IC_{50} (mg/ml)	Trolox equivalent (μg/g DW)	
***J. sabina* RM**	2.60 ±0.17	53.77 ±3.48	0.16 ±0.02	217.06 ±0.28	221.45 ±1.35
***J. sabina* SN**	1.64 ±0.01	85.57 ±0.21	0.13 ±0.01	267.15 ±0.35	303.99 ±1.16
***J. sabina* SPM**	1.93 ±0.01	72.58 ±0.32	0.15 ±0.04	231.53 ±0.41	261.27 ±5.31
***P. sylvestris* RM**	6.68 ±0.64	20.96 ±0.53	0.41 ±0.03	84.71 ±0.18	92.14 ±7.19
***P. sylvestris* SN**	7.28 ±0.55	19.23 ±0.55	0.51 ±0.04	68.10 ±0.14	75.29 ±0.39

Determination of the biopesticidal activity of obtained extracts and oils from *J. sabina* against Colorado Potato beetle (*Leptinotarsa decemlineata* Say) in the laboratory conditions

Ovicidal activity

It was established, that both 5.0% ethanolic extracts and 0.1% essential oils (from Moldova and Slovakia) in the laboratory condition possessed higher ovicidal activity against *L. decemlineata* (Table 3). In none of the variants of the *J. sabina* hatching of the larvae of the Colorado, beetle was observed. Whereas in the control hatching of the larvae reached 100% on the second day of the experiment. With a decrease in the concentration of alcohol extracts (from 0.1 to 20%), ovicidal activity significantly decreases – $LSD_{0.05}$ = 61.5; $p \leq 0.05$ (Figure 1).

Early, we found that at a concentration of 1.0–2.5% (dry solids), the ethanol extract of *J. sabina* exhibits low insecticidal activity in regard to the egg-laying of the Colorado potato beetle (Elisovetskaya, 2015). The new obtained data showed that needles of the *J. sabina* contain biologically active substances with ovicidal properties, which, however, are insufficient quite extracted with 96% ethanol. However, it was found that *J. sabina* essential oil is most promising as an ovicidal means against Coleopteran insects if it is used indoors, such as greenhouses, storages, etc.

Table 3 Insecticidal, antifeedant and ovicidal activity of plant extract and essential oil from *Juniperus sabina* L. against *Leptinotarsa decemlineata* Say in the laboratory condition, 2018

Variants		Concentration (dry residue for ethanolic extract) (%)	Insecticidal activity (%)		Antifeedant activity (point)		Ovicidal activity (%)
			imago	larvae of II-III instars	imago	larvae of II-III instars	
Control		–	0	0	5	5	0
			Ethanolic extract				
J. sabina	Slovakia	20.0	13.3	73.7	2–3	1	100
	Moldova	20.0	6.7	66.7	2–3	1	100
Essential oil							
J. sabina	Slovakia	0.1	53.3	100	1	1	100
	Moldova	0.1	26.7	100	1	1	100
		$LSD_{0.05}$ =15.4 *p* ≤0.05		$LSD_{0.05}$ =10.9 *p* ≤0.05			

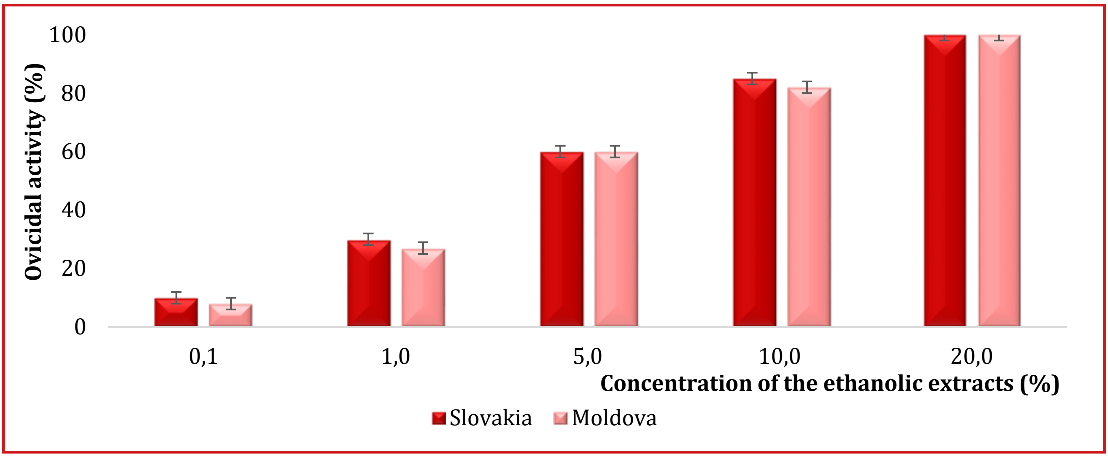

Figure 1 Ovicidal activity ethanolic extracts (from Moldova and Slovakia) depending on the concentration, against *Leptinotarsa decemlineata* Say in the laboratory condition ($LSD_{0.05}$ = 61.5; $p \leq 0.05$)

Insecticidal and antifeedant activity

As a result of laboratory testing, it was revealed that essential oils from *J. sabina* were more effective than ethanolic extracts against imagoes and larvae of potato beetle (Table 3). It was established that the character of extracts effects on insects is intestinal and insignificant contact. Essential oils have a pronounced contact action, as well as intestinal and „fumigant". The lowest insecticidal activity was observed by application of alcohol extracts against adult insects. In variants with essential oil, the death of larvae reached 100% in both variants

(Moldova and Slovakia). It was determined, that ethanolic extract and essential oil of *J. sabina* from Slovakia showed higher insecticidal properties against the Colorado beetle than the same from Moldova. Statistical analysis demonstrated, that the difference was significant only for variant with imago – between essential oils from Moldova and Slovakia ($LSD_{0.05}$ =15.4, $p \leq 0.05$). There was no significant difference between the other variants (except control). All plant preparations showed high antifeedant properties to the larvae and imago Colorado potato beetle, which were slightly lower for imago in ethanol extracts compared to essential oils (Table 3). Thus these extracts and essential oils showed high level insecticidal activity, combining contact, intestinal and fumigant action, against larvae Coleopterans, which coincided very well with our previous studies (Elisovetcaia et al., 2017; 2018a).

Repellent and deterrent activities

Our observation for *L. decemlineata* during the first hour after the accommodation in the Petri dishes showed that larvae in the experiment moved in the opposite direction from the feed and kept along the walls of dishes, trying not to approach the treatment leaves of the potato. They circled on the perimeter of the Petri dishes, sometimes approached to the treated feed, but, in this case, larvae immediately, very quickly, moving in the opposite direction. This fact indicates the presence of the repellent effect of extracts and essential oil from *J. sabina*. Comparative analysis showed that the control larvae moved to the feed in the first 5–7 seconds.

Summing up what has been said, it was determined, that mortality of imago and larvae *L. decemlineata* when using plant preparations from *J. sabina* consisted in average 6.7–53.3% and 66.7–100% respectively, ovicidal activity was 100%, antifeedant effect persisted at the level of 1–3 points for imago and 1 point for larvae. As well as were established repellent and deterrent activities for the extracts and essential oil from *J. sabina* relative to *L. decemlineata*. The highest insecticidal, antifeedant, repellent and deterrent properties against Colorado potato beetle were shown by essential oil *J. sabina* from Slovakia. These results are consistent with those obtained previously (Elisovetcaia et al., 2017) and prove the presence of identical properties in the plant *J. sabina*, regardless of the region of growth.

Determination of biopesticidal activity of obtained extracts and oils from *J. sabina* and *P. sylvestris* against wax month *Galleria mellonella* in the laboratory conditions

As a result of laboratory testing, it was revealed that extracts and essential oils from *J. sabina* were more effective against larvae *G. mellonella* than extracts and essential oils from *P. sylvestris* (Table 4).

Insecticidal activity

It was found that the greatest insecticidal effect was achieved in the variant with the dorsal application of essential oil from *J. sabina* – 65.0% mortality of larvae. Statistical analysis of the data showed that there is no significant difference between all the variants with extracts and essential oils from *P. sylvestris*. At the same time, in case of *J. sabina*, insecticidal activity significantly depends both on the method of extracting biologically active substances, and on the means of treatment – introduction extract or oil into the feed or topically on the dorsal field of the insects. It was established that biologically active substances contained in pine needles have a more intestinal effect than contact, while for *J. sabina* the exact opposite is true (Table 4).

Table 4 Effect of essential oil and ethanolic extract from *Juniperus sabina* L. and *Pinus sylvestris* L. on the survival of larvae, the formation of pupae molting of imago and on the feed consumption of the larvae *Galleria mellonella* Linnaeus

Variants	Mortality larvae (%)	Pupation (%)	Imago molted (%)	Mortality imago (%)	Average amount of feed consumption (larva/3 day) (g)
Control (0.5% Ethanol*)	0	100	100	0	0.0693 ±0.0006
Control (0.5% Ethanol)**	0	100	100	0	0.0690 ±0.0005
Control***	0	100	100	0	0.0696 ±0.0007
***Js* 0.1% Ethanolic extract ***	10.0	30.0	24.0	16.7	0,0126 ±0.0004
Js* 0.1% Ethanolic extract*	20.0	48.0	36.0	55.0	0,0252 ±0.0005
Js* Essential oil	20.0	15.0	24.0	66.7	0,0105 ±0.0003
Js* Essential oil*	65.0	15.0	8.0	50.0	0,0087 ±0.0002
Ps* 0.1% Ethanolic extract	13.3	46.7	46.7	0	0.0219 ±0.0004
Ps* 0.1% Ethanolic extract*	6.7	46.7	53.3	0	0.0183 ±0.0003
Ps* Essential oil	13.3	40.0	40.0	0	0.0213 ±0.0004
Ps* Essential oil*	6.7	46.7	53.3	0	0.0177 ±0.0005
	$LSD_{0.05}$ = 5.1, *p* ≤0.05			$LSD_{0.05}$ = 0.0042, *p* ≤0.05	

Notes: * treatment of the feed, 200 μkl; ** treatment of the dorsal field, 5 μkl; *** without total treatment

Antifeedant activity

As a result of the experiment it was established, that the extracts and essential oils from *J. sabina* and *P. sylvestris* have high antifeedant activity against larvae *G. mellonella*. Amount of feed consumption of the larvae wax moth in the laboratory conditions (during the first three days of the experiment) both when using extracts from *J. sabina* and *P. sylvestris*, and their essential oils, was significantly less than in the control variants (Table 4). There was no significant difference in the feed consumption between the different control variants. At the same time, it was noted that was a significant difference between the variants with extracts and oils from *J. sabina* and *P. sylvestris*. Thus, it was found that extract and essential oil from *J. sabina* more effectively reduced feed consumption than from *P. sylvestris*. It is also noted that between the means of treatment (insects or feed) the difference is not significant, except for the ethanol extract from *J. sabina* – the dorsal application was more effective. In other words, in the variant with topical application of essential oil from *J. sabina* to the dorsal field of insects the lowest feed consumption was observed (Table 4). Earlier, Chinese researchers (Gao et al., 2004) have proved the high insecticidal and antifeedant activities of deoxypodophyllotoxin (DPP), isolated from a petroleum ether extract of *Juniperus sabina*, against lepidopteran larvae *Pieris rapae* L. (Lepidoptera: Pieridae) At 0.015–0.100 g/litre, DPP showed insecticidal activity, with an LC_{50} of 0.020 g/litre. Antifeedant activity against fifth-instar larvae of *P. rapae* was at 0.05–1.00 g/litre and its AFC_{50} (concentration for 50% antifeedant activity) values at 12 and 48 h were 0.170 and 0.060 g/litre, respectively. Our studies showed that ethanol extracts and essential oils from *J. sabina* also exhibit pronounced insecticidal and antifeedant properties against another representative of Lepidoptera – *G. mellonella* (Pyralidae). However, it was found that the level of biopesticidal activity significantly depends on the extraction method and ontogenetic phase of the pest development. Differences in the activity level of extracts from *J. sabina* confirm the theory that secondary plant metabolites have a selective effect and affect multiply on the various species of insects.

Repellent and deterrent activities

The repellent activity of extracts and essential oils from *J. sabina* and *P. sylvestris* was confirmed by observing the behavior of larvae *G. mellonella* during the first hours after the accommodation in the Petri dishes. Insects moved in the opposite direction from the feed and circled around the feed for a long time, not approaching it. The time between replanting and the start of nutrition in the experimental variants was 9–40 (and more) times higher than the control and ranged from 90–120 seconds to 3–5 minutes. New data are consistent with our previous data (Elisovetcaia and Brindza, 2018).

The deterrent properties of the extracts and oils from *J. sabina* and *P. sylvestris* were manifested in suppressing the nutrition of the wax moth larvae, reducing the number of the pupated larva, molted imago, as well as in suppressing oviposition of the adults *G. mellonella* in comparison with the control (Table 4). Feed consumption in the experimental variants with extracts and essential oils from *J. sabina* and *P. sylvestris* was 2.75–6.6 times lower than in control; pupation decreased by 2.1–6.7 times, and the molting of imago – by 1.9–8.0 times. It was observed that essential oils had also deterrent effect on egg-laying of the wax moth – the number of eggs laid in the variants treated with extracts and essential oils was 25–100% lower than in the control.

Thus, all tested plant preparations from Coniferous (*J. sabina* and *P. sylvestris*) showed high insecticidal, antifeedant, repellent and deterrent properties against Lepidopterans (*G. mellonella*), as well as ethanolic extracts and essential oil from *J. sabina* and against Coleopterans (*L. decemlineata*). These data significantly supplement the previously obtained results (Elisovetcaia and Brindza, 2018).

Conclusions

For the first time was carried out a comparative analysis of the antioxidant and biopesticidal activity of two species of conifers – *Juniperus sabina* and *Pinus sylvestris*, growing in the Republic of Moldova (RM) and Slovak Republic, Nitra (SN) and Pianiny mountains (PM). The ethanolic extracts of *J. sabina* from Nitra showed the highest antioxidant activity against both DPPH and peroxyl free radicals. All tested samples conform to diminution of antioxidant activity were presented by followed order: *J. sabina* SN > *J. sabina* PM > *J. sabina* RM> *P. sylvestris* RM ≥ *P. sylvestris* SN (DPPH – 85.57 ±0.21 > 72.58 ±0.32 > 53.77 ±3.48 > 20.96 ±0.53 >19.23 ±0.55 and peroxyl free radicals – 267.15 ±0.35 > 231.53 ±0.41 > 217.06 ±0.28 > 84.71 ±0.18 > 68.10 ±0.14). Moreover, it was established that both ethanolic extracts and essential oils from *J. sabina* possessed high biopesticidal efficiency against *Leptinotarsa decemlineata* and *Galleria mellonella*. Ovicidal activity of the extracts and essential oils from *J. sabina* against *L. decemlineata* reached 100%, mortality of imago and larvae consisted on average 6.7–53.3 and 66.7–100% respectively. The antifeedant effect of all plant preparations persisted at the level of 1–3 points (foliage ingestion did not exceed 5–25%). It was established that the essential oils were more effective across the board than ethanolic extracts. It was revealed that against larvae *G. mellonella* extracts and essential oils from *J. sabina* were more effective than from *P. sylvestris*. The greatest insecticidal effect was achieved in the variant with the dorsal application of essential oil from *J. sabina* – 65.0% mortality of larvae. The extracts and essential oils from *J. sabina* and *P. sylvestris* have a high antifeedant, repellent and deterrent activities, that expressed in the suppressing the nutrition of the wax moth larvae, reducing the number of pupated larva, molted imago, as well as in suppressing oviposition of the adults *G. mellonella* in comparison with the control. Feed consumption in the experimental variants with extracts and essential oils from *J. sabina* and *P. sylvestris* was 2.75–6.6 times lower than in control; pupation decreased by 2.1–6.7 times, and the molting of imago – by 1.9–8.0 times. Also the number of eggs laid in the variants treated with extracts and essential oils was 25–100% lower than in the control.

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