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Full Length Research Paper

Screening of Eurasian plants for insecticidal and growth inhibition activity against *Spodoptera littoralis* larvae

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This paper presents the results of a screening of plant extracts obtained from 134 plant species of the Eurasian region for chronic toxicity and larval inhibition in *Spodoptera littoralis* larval growth. The extracts from *Ailanthus altissima*, *Ajuga chamaepitys*; *Ajuga reptans*, *Angelica archangelica*, *Artemisia campestris*, *Buphtalmum salicifolium*, *Camellia sinensis*, *Chenopodium bonus-henricus*, *Eupatorium cannabinum*, *Foeniculum vulgare*, *Lythrum salicaria, Lythrum virgatum*, *Mentha arvensis*, *Mentha longifolia*, *Mentha suaveolens*, *Potentila argentea*, *Potentila fruticosa*, *Seseli pallasii* and *Vincetoxicum hirundinaria* were selected, which caused both 100% larval mortality and growth inhibition higher than 75% after application of 15 mg dose of the extract in 1 g of food. Lethal doses and the effect of LD_{50} on growth inhibition and antifeedant were estimated in order to determine the differences in efficiency of the selected extracts based on the mortality results, the extract from *A. archangelica* seeds could be chosen as the most efficient one for its LD_{50} was significantly lower (0.4 mg/g) compared to the other extracts.

Key words: *Spodoptera littoralis*, plant extracts, botanical insecticides, antifeedancy, acute toxicity.

INTRODUCTION

Pesticides are used for the protection of food, fiber, human health and comfort around the world. However, intensive use of synthetic insecticides to control insect pests had led to many problems such as pest resistance and resurgence, negative effects on non-target organisms including humans, and negative environmental impacts (Ecobichon, 2001). These effects have provided the impetus for the development of alternatives, including botanical insecticides. Use of botanical insecticides is one of plant protection alternatives, generally considered as safe for the environment and health (Pavela, 2007; Dubey, 2010). Significant efforts are thus devoted at present to searching for new, highly efficient plant extracts, which would be suitable for the development of botanical insecticides (Dubey, 2010; Pavela, 2010b).

The use of plants as traditional protectants of plant products is an old practice used all over the world. Our ancestors were quite successful in exploring and exploiting this natural treasure. The documented use of plant extracts and powdered plant parts as insecticides goes back at least as far as the Roman Empire. There are reports of the use of pyrethrum (*Tanacetum cinerariaefolium*) already in 400 B.C. *T. cinerariaefolium* extracts met with such a success that they remain in use even now, representing, together with botanical insecticides based on extracts from *Azadirachta indica* Juss, *Pongamia pinnata* and some essential oils, the largest share of the world market for botanical pesticides. However, their production and thus also their use are limited due to the lengthy production time for the plant material, lasting at least one year. This is why new plant species have been sought that could be used to produce botanical insecticides (Pavela, 2007; Dubey, 2010).

Central European and Russian flora is very rich in plant species. Many of the plants have been favourite in folk medicine, cosmetics, food industry, and in other industries (Brunneton, 1999). As demonstrated earlier, plants of these regions contain compounds that exhibit insecticidal (Pavela, 2005, 2006, 2008, 2009a, b, 2010a), fungicidal (Zabka et al., 2009) and bactericidal (Kokoskova and Pavela, 2007) effects, and thus, these plants provide considerable prospects in the sense that they may become as a source to develop new and environmentally safe botanical pesticides. Studies on

insecticidal efficiency of compounds obtained from plants are very important to determine the further direction of research and development of new botanical insecticides. Environmentally safe compounds or extracts should be considered to keep finding such plant species whose extracts do not cause primarily high acute toxicity, but they rather exhibit a good effect on reduced consumption of food intake, growth inhibition, and chronic toxicity of phytophagous pests. Botanical insecticides of such extracts provide the higher chance of being friendly on non-target organisms, predominantly to natural enemies of the pests, which are important from the environmental point of view (Kaushik et al., 2009; Pavela, 2010b; Rattan, 2010). The noctuid *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae) is a most important polyphagous pest, widely distributed in Africa and Mediterranean Europe (Pineda et al., 2006). Commonly, the control of this pest has largely been depending on the use of neurotoxic insecticides including chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids (Baldwin and Graves, 1991; Saleem et al., 2008; Ahmad et al., 2009). However, the control achieved is not successful because of the insect's high capacity to develop resistance toward the majority of these compounds (Ghoneim et al., 2002; Abo et al., 2005). Therefore, we chose precisely this polyphagous pest for our experiments.

Plant material for the experiments was selected based on an ethnobotanical exploration undertaken in the previous period. In particular, medicinal plant species used in popular medicine were selected, since one can expect them to be safe for human health (Pavela, 2009c). This paper presents the results from the screening of plant extracts obtained from 134 plant species of the Eurasian region for chronic toxicity and inhibition of *S. littoralis* larval growth. Growth inhibition and antifeedant effects after application of lethal doses causing chronic toxicity were subsequently determined for the most efficient extracts. Those plant extracts were selected for the tests which caused not more than 30% acute toxicity (assessed after 24 h and after application of the dose 300 µg/larvae) in preliminary tests.

MATERIALS AND METHODS

Plant material

Fresh plant materials from each of the selected species (Table 1) were collected in 2009. Voucher specimens of all the plant species studied were deposited in the respective herbaria of our institute. The plant material was shade-dried (40°C).

Extraction

The plant materials were pulverized and extracted using 100% pure methanol during 48 h at the laboratory temperature (ratio plants: methanol; 1:10). The crude extracts were separately filtered and evaporated under reduced pressure in a rotary evaporator.

Insects

S. littoralis: Bioassays were conducted using larvae of the tobacco cutworm, *S. littoralis*, obtained from an established laboratory colony (> 20 generations; out-crossed once). The larvae fed on an artificial insect diet (Stonefly Industries, Bryan, TX, USA); adults fed on a 10% honey solution and were able to oviposit on filter paper. The colonies were reared at 25 ± 1°C and a 16:8 (L:D) photoperiod. This experiment was performed with pre-weighed, newly-moulted (0–6 h after ecdysis) 4th instar larvae.

Bioassays

Toxicity

Chronical toxicity of extracts (Table 1), measured as mortality after 5 days, was determined by oral application to early fourth instars larvae *S. littorralis*. Considering the high number of tested plants, at first the extracts were subjected to a basic test in order to select the most efficient extracts. The maximum dose 15.0 mg in 1.0 g of diet (this dose was determined based on our experience as an approximate upper limit of economic rentability, and it corresponds to the dose of about 3 kg of the extract/ ha) was applied with the aim to determine chronic toxicity of the extracts. For example, 150 mg of an extract was stirred up in 7.0 ml of water, and 3.0 g of dry artificial insect diet (Stonefly Industries, Bryan, TX, USA) was added after the extract dissolved, to prepare 10 g of contaminated diet. The mixture thus obtained was thoroughly homogenized by stirring (mechanical agitator, 300 RPM, stirring time 5 min). Diet with water only was used for the control larvae.

Thus, prepared diet was administered *ad libitum* to new larvae of *S. littoralis*, 4th instar. Larval mortality was assessed 5 days after establishing the experiment. The extracts caused 100.0% mortality were chosen for determining lethal doses. Diets contaminated with extracts in five doses (12, 6, 3, 1 and 0.1 mg/g) were administered to *S. littoralis* larvae, in order to determine lethal doses; the diet was prepared identically as described earlier. Four replications of 20 larvae were tested per dose. All larvae from each replicate were transferred in plastic boxes (10 × 10 × 7 cm). The boxes were placed for 5 days in a growth chamber (L16:D8, 25°C). Death was recorded when the larvae did not respond to prodding with forceps.

Effect on the larval growth

Diet containing extracts in the dosage 15 mg/g was administered to *S. littoralis* larvae, in order to determine extract efficiency on larval growth. The diet was prepared identically as described earlier. Newly emerged 4th instar were weighed and placed individually in Petri dishes (6 cm in diameter). The contaminated diet was given to the larvae *ad libitum* for 3 days. Subsequently, the larvae were weighed, and the growth inhibition index was calculated based on the determined weight increments according to the formula: GI (%) = 100-[(T/C)×100)], where C and T are weight increments of the larvae that consumed control and contaminated diet, respectively. For the sake of better orientation, the extracts were divided in four groups based on the range of calculated efficiency, where: + is GI lower than 20%; ++ is GI 21 to 40%; +++ is GI 41 to 70% and ++++ is GI higher than 71%.

GI for extracts causing 100.0% mortality was determined identically as described previously; however, diet contaminated with dosage corresponding to the estimated LD_{50} was given to the larvae. Twenty new larvae of the 4th instar were always tested for every dose. The experiment was placed in the growth room (L16:D8, 25°C). The experiment was replicated 3 times.

Table 1. Plants used in this study, their part used, origin, voucher references and yield of extracts.

Species	Family	Plant part assayed	Yield (%)	Voucher references	Origin
Acer campestre L.	Aceraceae	Leaves	9.8	9182	Prague, Czech Republic
Acer capillipes Maxim.	Aceraceae	Leaves	12.5	9181	Prague, Czech Republic
Acer platanoides L.	Aceraceae	Leaves	9.3	9183	Prague, Czech Republic
Acinos arvensis (Lam.) Dandy	Lamiaceae	Stem	8.4	990	Vranov nad Dyjí, Czech Republic
Aegopodium podagraria L.	Apiaceae	Stem	5.6	9138	Dobré, Czech Republic
Achillea ageratum L.	Asteraceae	Stem	11.8	9155	Prague, Czech Republic
Achillea collina Heimerl	Asteraceae	Stem	7.0	9149	Prague, Czech Republic
Achillea nobilis L.	Asteraceae	Stem	10.9	9141	Prague, Czech Republic
Ailanthus altissima (Mill.) Swingle	Simaroubaceae	Leaves	15.3	907	Chomutov, Czech Republic
Ajuga chamaepitys (L.) Schreber	Lamiaceae	Stem	14.8	9142	Prague, Czech Republic
Ajuga reptans L.	Lamiaceae	Stem	6.1	951	Prague, Czech Republic
Anethum graveolens L.	Apiaceae	Stem	12.4	9157	Prague, Czech Republic
Angelica archangelica L.	Apiaceae	Roots	4.3	958	Hodonín, Czech Republic
Anthemis tinctoria L.	Asteraceae	Flower	5.1	945	Prague, Czech Republic
Arctium lappa L.	Asteraceae	Stem	7.3	917	Ljulin Mountain, Bulgaria
Artemisia abrotanum L.	Asteraceae	Stem	8.3	919	Prague, Czech Republic
Artemisia absinthum L.	Asteraceae	Stem	5.3	952	Prague, Czech Republic
Artemisia campestris L.	Asteraceae	Stem	6.9	910	Krasnodarskiy region, Russia
Asarum europaeum L.	Arisrolochiaceae	Stem	7.5	9122	Vranov nad Dyjí, Czech Republic
Astragalus glycyphylloides DC.	Fabaceae	Stem	9.2	9109	Vranov nad Dyjí, Czech Republic
Astragalus glycyphyllos L.	Fabaceae	Roots	5.1	963	Hodonín, Czech Republic
Astragalus glycyphyllos L.	Fabaceae	Stem	9.1	9164	Prague, Czech Republic
Astragalus chinensis L. f.	Fabaceae	Stem	7.7	944	Prague, Czech Republic
Astrantia major L.	Apiaceae	Stem	6.8	9104	Blatnica, Slovak Republic
Balsamita major Desf.	Asteraceae	Stem	7.5	930	Prague, Czech Republic
Borago officinalis L.	Boraginaceae	Stem	4.5	9175	Prague, Czech Republic
Bryonia dioica Jacq.	Cucurbitaceae	Stem	6.8	940	Prague, Czech Republic
Buddleja davidii Franch.	Buddlejaceae	Stem	21.0	9165	Prague, Czech Republic
Buphtalmum salicifolium L.	Asteraceae	Stem	8.1	9112	Blatnica, Slovak Republic
Bupleurum falcatum L.	Apiaceae	Stem	9.6	9118	Vranov nad Dyjí, Czech Republic
Camellia sinensis (L.) Kuntze (černý)	Theaceae	Leaves	8.0	987	Prague, Czech Republic
Camellia sinensis (L.) Kuntze (zelený)	Theaceae	Leaves	13.0	986	Prague, Czech Republic
Campanula rapunculoides L.	Campanulaceae	Stem	7.2	9106	Znojmo, Czech Republic
Campanula rotundifolia L.	Campanulaceae	Stem	9.8	9123	Vranov nad Dyjí, Czech Republic

Table 1. Contd.

Carthamnus lanatus L.	Asteraceae	Stem	7.9	916	Ljulin Mountain, Bulgaria
Centaurea cyanus L.	Asteraceae	Stem	6.3	988	Vranov nad Dyjí, Czech Republic
*Centaurea elatio*r (Gaud.) Hayek	Asteraceae	Stem	6.7	9107	Blatnica, Slovak Republic
Cichorium intybus L.	Asteraceae	Stem	5.9	911	Krasnodarskiy region, Russia
Clematis vitalba L.	Ranunculaceae	Stem	8.2	912	Ljulin Mountain, Bulgaria
Clinopodium vulgare L.	Lamiaceae	Stem	8.4	9103	Blatnica, Slovak Republic
Cola nitida (Vent.) A. Chev.	Sterculiaceae	Seeds	2.7	961	Hodonín, Czech Republic
Colymbada scabiosa (L.) Holub	Asteraceae	Stem	9.2	997	Blatnica, Slovak Republic
Daucus carota L.	Apiaceae	Stem	8.1	9151	Prague, Czech Republic
Dracocephalum moldavica L.	Lamiaceae	Stem	8.0	9167	Prague, Czech Republic
Dracocephalum moldavicum L.	Lamiaceae	Stem	5.5	929	Prague, Czech Republic
Echinacea pallida (Nutt.)	Asteraceae	Roots	5.1	931	Valtice, Czech Republic
Echinacea purpurea (L.) Moench	Asteraceae	Flower	8.9	956	Prague, Czech Republic
Echinops sphaerocephalus L.	Asteraceae	Stem	8.9	915	Krasnodarskiy region, Russia
Eupatorium cannabinum L.	Asteraceae	Stem	6.5	9108	Blatnica, Slovak Republic
Falcaria vulgaris Bernh.	Apiaceae	Stem	7.1	9105	Znojmo, Czech Republic
Fallopia sachalinensis (F.Schmidt)	Polygonaceae	Stem	4.2	904	Chomutov, Czech Republic
Ferula assa-foetida L.	Apiaceae	Stem	3.5	926	Prague, Czech Republic
Filipendula ulmaria (L.) Maxim.	Rosaceae	Stem	7.8	957	Prague, Czech Republic
Foeniculum vulgare Mill.	Apiaceae	Seeds	5.9	9161	Prague, Czech Republic
Galega officinalis L.	Fabaceae	Stem	14.8	9168	Prague, Czech Republic
Galeobdolon argentatum Smejkal	Lamiaceae	Stem	15.1	9137	Vranov nad Dyjí, Czech Republic
Galium sylvaticum L.	Rubiaceae	Stem	5.2	9119	Vranov nad Dyjí, Czech Republic
Grindelia camporum Greene	Asteraceae	Stem	6.9	939	Prague, Czech Republic
Grindelia hirsutula Hook. & Arn.	Asteraceae	Stem	7.4	992	Olomouc, Czech Republic
Grindelia squarrosa (Pursh) Dunal	Asteraceae	Stem	13.4	995	Olomouc, Czech Republic
Grindelia stricta subsp. oregana D.D. Keck	Asteraceae	Stem	10.2	993	Olomouc, Czech Republic
Grindelia stricta subsp. venulosa (Jeps.) D.D. Keck	Asteraceae	Stem	11.2	994	Olomouc, Czech Republic
Helianthemum grandiflorum (Wahlenb.) Holub	Cistaceae	Stem	8.8	9125	Vranov nad Dyjí, Czech Republic
Hepatica nobilis Schreb.	Ranunculaceae	Stem	8.4	9117	Vranov nad Dyjí, Czech Republic
Heracleum sphondylium L.	Apiaceae	Stem	8.4	9135	Dobré, Czech Republic
Hypericum montanum L.	Hypericaceae	Stem	16.1	991	Blatnica, Slovak Republic
Hyssopus seravschanicus (Dub.) Pazij	Lamiaceae	Stem	11.0	9152	Prague, Czech Republic
Chaerophyllum hirsutum L.	Apiaceae	Stem	15.5	9124	Blatnica, Slovak Republic
Chenopodium bonus-henricus L.	Chenopidiaceae	Roots	15.0	960	Hodonín, Czech Republic

Table 1. Contd.

Inula magnifica Lipsky	Asteraceae	Stem	11.8	9158	Prague, Czech Republic
Jatropha curcas L.	Euphorbiaceae	Leaves	6.5	947	Prague, Czech Republic
Lathyrus pratensis L.	Fabaceae	Stem	6.2	999	Blatnica, Slovak Republic
Lathyrus tuberosus L.	Fabaceae	Stem	13.5	9120	Znojmo, Czech Republic
Lavandula angustifolia Mill.	Lamiaceae	Stem	9.4	9176	Prague, Czech Republic
Lavandula canariensis Mill.	Lamiaceae	Stem	6.2	9156	Prague, Czech Republic
Lembotropis nigricans L. Griseb.	Fabaceae	Stem	7.8	998	Blatnica, Slovak Republic
Leuzea carthamoides (Willd.) DC.	Asteraceae	Roots	8.2	932	Valtice, Czech Republic
Leuzea carthamoides (Willd.) DC.	Asteraceae	Seeds	18.3	933	Valtice, Czech Republic
Levisticum officinale W. D. J. Koch	Apiaceae	Roots	5.8	966	Hodonín, Czech Republic
Lobelia siphilitica L.	Lobelioideae	Stem	5.6	937	Prague, Czech Republic
Lotus corniculatus L.	Fabaceae	Stem	7.3	9139	Dobré, Czech Republic
Lythrum salicaria L.	Lythraceae	Stem	8.4	9126	Blatnica, Slovak Republic
Lythrum salicaria L.	Lythraceae	Stem	9.9	924	Prague, Czech Republic
Lythrum virgatum L.	Lythraceae	Stem	9.6	9163	Prague, Czech Republic
Medicago falcata L.	Fabaceae	Stem	9.2	9113	Znojmo, Czech Republic
Melilotus albus Medik.	Fabaceae	Stem	8.9	953	Prague, Czech Republic
Melilotus albus Medik.	Fabaceae	Stem	10.7	9140	Dobré, Czech Republic
Mentha arvensis L.	Lamiaceae	Stem	6.2	9150	Prague, Czech Republic
Mentha longifolia (L.) L.	Lamiaceae	Stem	9.2	9154	Prague, Czech Republic
Mentha suaveolens Ehrh.	Lamiaceae	Stem	16.9	9153	Prague, Czech Republic
Nepeta pannonica L.	Lamiaceae	Stem	8.5	9148	Prague, Czech Republic
Ononis arvensis L.	Fabaceae	Stem	12.6	9145	Prague, Czech Republic
Ononis spinosa L.	Fabaceae	Stem	6.3	9101	Blatnica, Slovak Republic
Onopordon acanthium L.	Asteraceae	Stem	9.3	914	Krasnodarskiy region, Russia
Origanum dictamnus L.	Lamiaceae	Stem	9.4	9173	Prague, Czech Republic
Origanum vulgare L.	Lamiaceae	Stem	14.0	9177	Prague, Czech Republic
Origanum vulgare L.	Lamiaceae	Stem	7.4	9178	Prague, Czech Republic
Origanum vulgare L.	Lamiaceae	Stem	9.5	9179	Prague, Czech Republic
Orlaya grandiflora (L.) Hoffm.	Apiaceae	Stem	11.4	9166	Prague, Czech Republic
Panax ginseng C. A. Mey	Araliaceae	Roots	8.1	965	Hodonín, Czech Republic
Petasites hybridus L.	Asteraceae	Rhizome	6.2	962	Hodonín, Czech Republic
Phacelia tanacetifolia Benth.	Hydrophyllaceae	Stem	7.8	9136	Dobré, Czech Republic
Physalis alkekengi L.	Solanaceae	Stem	6.9	935	Prague, Czech Republic
Plantago lanceolata L.	Plantaginaceae	Stem	18.6	9143	Prague, Czech Republic
Polygonum aviculare L.	Polygonaceae	Stem	6.9	9100	Znojmo, Czech Republic

Table 1. Contd.

Populus nigra L.	Salicaceae	Leaves	4.5	959	Hodonín, Czech Republic
Potentila argentea L.	Rosaceae	Stem	10.5	9116	Vranov nad Dyjí, Czech Republic
Potentilla anserina L.	Rosaceae	Stem	7.3	9171	Prague, Czech Republic
Potentilla fruticosa L.	Rosaceae	Stem	21.6	9170	Prague, Czech Republic
Potentilla hirta L.	Rosaceae	Stem	2.1	9162	Prague, Czech Republic
Potentilla reptans L.	Rosaceae	Stem	12.2	9172	Prague, Czech Republic
Pyrethrum parthenium (L.) Sm.	Asteraceae	Stem	12.3	9144	Prague, Czech Republic
Reynoutria × bohemica Chrtek & Chrtková	Polygonaceae	Leaves	11.8	901	Chomutov, Czech Republic
Rubia tinctorum L.	Lamiaceae	Stem	8.5	942	Prague, Czech Republic
Rumex acetosella L.	Polygonaceae	Stem	4.6	9111	Blatnica, Slovak Republic
Salvia glutinosa L.	Lamiaceae	Stem	4.1	941	Prague, Czech Republic
Salvia officinalis L.	Lamiaceae	Stem	14.0	9146	Prague, Czech Republic
Saponaria officinalis L.	Caryophyllaceae	Stem	13.8	9114	Znojmo, Czech Republic
Saponaria officinalis L.	Caryophyllaceae	Stem	6.3	943	Prague, Czech Republic
Scrophularia nodosa L.	Scrophulariaceae	Stem	5,6	936	Prague, Czech Republic
Securigera varia (L.) Lassen	Fabaceae	Stem	13.8	9110	Vranov nad Dyjí, Czech Republic
Sedum rosea (L.) Scop.	Crassulaceae	Flower	11.3	950	Prague, Czech Republic
Senecio umbrosus Waldst. et Kit.	Asteraceae	Stem	8.3	996	Blatnica, Slovak Republic
Seseli pallasii Besser	Apiaceae	Stem	6.1	927	Prague, Czech Republic
Schisandra chinensis (Turcz.) Baill	Schisandraceae	Leaves	8.7	928	Prague, Czech Republic
Silene vulgaris L.	Caryophyllaceae	Stem	6.7	9115	Vranov nad Dyjí, Czech Republic
Silphium perfoliatum L.	Asteraceae	Leaves	8.7	905	Chomutov, Czech Republic
Stachys byzantina K.Koch	Lamiaceae	Stem	11.2	938	Prague, Czech Republic
Stachys palustris L.	Lamiaceae	Stem	8.0	9160	Prague, Czech Republic
Stachys recta L.	Lamiaceae	Stem	8.3	9147	Prague, Czech Republic
Stachys sylvatica L.	Lamiaceae	Stem	5.6	989	Vranov nad Dyjí, Czech Republic
Teucrium botrys L.	Lamiaceae	Stem	4.6	9169	Prague, Czech Republic
Teucrium capitatum L.	Lamiaceae	Stem	9.7	9174	Prague, Czech Republic
Teucrium hircanicum L.	Lamiaceae	Stem	12.6	946	Prague, Czech Republic
Teucrium chamaedrys L.	Lamiaceae	Stem	8.3	925	Prague, Czech Republic
Teucrium chamaedrys L.	Lamiaceae	Stem	10.4	9102	Blatnica, Slovak Republic
Thymus alpestris A. Kern.	Lamiaceae	Stem	10.4	9121	Blatnica, Slovak Republic
Thymus fragrantissimus Samen.	Lamiaceae	Stem	3.6	920	Prague, Czech Republic
Thymus serphyllum L.	Lamiaceae	Stem	2.3	921	Prague, Czech Republic
Trigonella foenum-graecum L.	Fabaceae	Seeds	7.5	985	Prague, Czech Republic
Valeriana officinalis L.	Valerianaceae	Roots	11.7	9180	Prague, Czech Republic

Table 1. Contd.

Verbena hirta Spreng.	Verbenaceae	Stem	5.2	955	Prague, Czech Republic
Vincetoxicum hirundinaria Medik.	Asclepiadaceae	Stem	8.2	934	Prague, Czech Republic
Withania somnifera L.	Solanaceae	Roots	5.2	964	Hodonín, Czech Republic

Antifeedant activity

The no-choice test was chosen to determine antifeedant activity, since its design was almost closely approaches to practical application (Koul, 2005). *S. littoralis* larvae were left with no food before the experiment, always for 3 h. The experiment itself was done in Petri dishes (9 cm in diameter). Damp filter paper was laid on the bottom of the dishes, and 4 disks, 1.5 cm in diameter and prepared using cork borer from tomatoes leaves, were always placed on the filter paper. The leaf disks were submersed in a solution with the most efficient extracts dissolved in water always for 5 to 10 s. The doses were determined separately for every extract, and corresponded to the estimated LD_{50} values.

Disks to which only the water had been applied were used as the control. After application, the leaf disks were left at rest for approximately 10 min to allow the solvent to evaporate. Afterwards, 2 starved larvae of *S. littoralis* were placed into the centre of every dish. The entire experiment was done in 15 repetitions. The experiment was terminated when the control larvae had consumed approximately 90% of the leaf disks (about 6 to 10 h, and 25°C). The area of the leaf disks consumed by larvae was then assessed and compared with control disks by using a screener software program (ABBYY FineReader 10) to determine antifeedant activity. The following could be calculated based on obtained data: feeding deterrence index FDI (%) = 100[(C-T)/(C + T)], where C and T are the control and treated leaf consumed by the insect (Koul, 2005).

Statistical analysis

Doses causing 50% (LD_{50}) mortality including corresponding values within a 95% confidence limit (CI_{95}), were estimated using Probit analysis. ANOVA was performed on the arcsine-transformed $\sqrt{(x/100)}$ percentage GI and FDI. Differences between treatment means were analysed using the Tukey's HSD test ($P<0.05$) (Abbott, 1925; Finney, 1971; SAS, 2000).

RESULTS

Effects of the extracts on chronic toxicity

S. littoralis larval mortality caused by extracts applied in food is shown in Table 2. Nineteen of 134 extracts exhibited the highest efficiency causing 100% mortality of the larvae. Other 17 extracts showed efficiency ranging between 50 to 99% of mortality, and 76 extracts caused relatively low mortality ranging between 10 to 50%. Only 22 extracts can be assessed as non-toxic for *S. littoralis* larvae as they caused mortality lower than 10%. The most efficient extracts obtained from *Ailanthus altissima*, *Ajuga chamaepitys*; *Ajuga reptans*, *Angelica archangelica*, *Artemisia campestris*, *Buphtalmum salicifolium*, *Camellia sinensis*, *Chenopodium bonus-henricus*, *Eupatorium cannabinum*, *Foeniculum vulgare*, *Lythrum salicaria, L. virgatum*, *Mentha arvensis*, *Mentha longifolia*, *Mentha suaveolens*, *Potentila argentea*, *Potentila fruticosa*, *Seseli pallasii* and *Vincetoxicum hirundinaria*, were selected for determining the lethal doses.

Effects of the extracts on larval growth

The efficiency of extracts on *S. littoralis* larval growth inhibition is presented in Table 2. Most of the tested extracts significantly inhibited larval growth compared to the control. The mean larval weight increment in the control during the experiment was 164.3 ± 8.2 mg/larvae. Thirty three extracts showed the most significant inhibition where GI higher than 71% was found. GI between 41 to 70% was found for 31 extracts and 20 to 40% for 27 extracts. Only 43 extracts inhibited larval growth compared to the control by less than 20%.

Effect of lethal doses on larval growth and on food intake

Lethal doses were determined for the selected 19 extracts (Table 3) based on primitively chronic test. Significantly the lowest lethal dose (0.4 mg/g) was determined for the extract of *A. archangelica* seeds. Extracts from *L. salicaria* stem and *Camellia sinensis* leaves followed with LD_{50} 2.3 and 2.6 mg/g, respectively. Considerable efficiency was found also for extracts from *P. argentea*, *M. arvensis*, *M. longifolia*, *A. reptans* and *A. altissima* where lethal doses between 3.3 to 4.8 mg/g were estimated. Lethal doses higher than 5 mg/g were estimated for the other 11 extracts. All extracts inhibited larval growth compared to the control (Table 3). The mean larval weight increment in the control during the experiment was 122.9 ± 5.2 mg/larvae. The highest growth inhibition was shown by extracts from *L. virgatum*, *L. salicaria*, *A. altissima*, *P. fruticosa*

Table 2. Chronic mortality and growth inhibition activity of plant extracts against larvae of *S. littoralis* after exposure maximal dose 15 mg/g of diet.

Species	Mortality*	Growth inhibitory effects**
Acer campestre	73.5 ±8.7	++++
Acer capillipes	69.9 ±12.5	+++
Acer platanoides	58.3 ±15.3	++++
Acinos arvensis	18.6 ±5.9	++
Aegopodium podagraria	5.6 ±2.3	+
Achillea ageratum	39.2 ±5.5	+++
Achillea collina	25.3 ±8.5	++
Achillea nobilis	56.9 ±9.8	+++
Ailanthus altissima	100.0 ±0.0	++++
Ajuga chamaepitys	100.0 ±0.0	++++
Ajuga reptans	100.0 ±0.0	++++
Anethum graveolens	75.9 ±6.7	++
Angelica archangelica	100.0 ±0.0	+++
Anthemis tinctoria	0.0 ±0.0	+++
Arctium lappa	13.3 ±3.9	++
Artemisia abrotanum	50.1 ±7.9	++++
Artemisia absinthum	38.9 ±5.2	+
Artemisia campestris	100.0 ±0.0	++++
Asarum europaeum	68.9 ±12.7	++++
Astragalus glycyphylloides	5.2 ±3.1	+
Astragalus glycyphyllos	32.5 ±6.5	+
Astragalus chinensis	0.0 ±0.0	++
Astrantia major	65.5 ±5.6	+++
Balsamita major	15.6 ±5.8	+++
Borago officinalis	12.8 ±7.6	+
Bryonia dioica	8.9 ±5.2	+
Buddleja davidii	28.9 ±7.2	+++
Buphtalmum salicifolium	100.0 ±0.0	++++
Bupleurum falcatum	42.8 ±11.6	++++
Camellia sinensis	100.0 ±0.0	++++
Campanula rapunculoides	48.6 ±2.9	+
Campanula rotundifolia	42.5 ±7.8	++
Carthamnus lanatus	15.2 ±7.2	++
Centaurea cyanus	25.7 ±3.5	++
Centaurea elatior	38.6 ±5.3	++
Chaerophyllum hirsutum	39.8 ±7.5	+++
Chenopodium bonus-henricus	100.0 ±0.0	++++
Cichorium intybus	0.0 ±0.0	++
Clematis vitalba	0.0 ±0.0	+++
Clinopodium vulgare	28.3 ±5.9	+++
Cola nitida	49.7 ±5.2	++
Colymbada scabiosa	11.5 ±3.4	++
Daucus carota	18.2 ±5.6	+
Dracocephalum moldavica	39.9 ±8.6	++
Dracocephalum moldavicum	0.0 ±0.0	+++
Echinacea pallida	18.5 ±7.2	++
Echinacea purpurea	25.7± 6.5	+
Echinops sphaerocephalus	36.7±4.2	++
Eupatorium cannabinum	100.0±0.0	++++
Falcaria vulgaris	34.3±3.3	+

Table 2. Contd.

Fallopia sachalinensis	20.3±5.3	++
Ferula assa-foetida	17.5±2.9	+
Filipendula ulmaria	69.5±7.3	++++
Foeniculum vulgare	100.0±0.0	++++
Galega officinalis	5.8±3.3	++
Galeobdolon argentatum	10.5±6.2	+
Galium sylvaticum	18.5±6.9	+
Grindelia camporum	21.5±3.5	+
Grindelia hirsutula	18.5±6.8	+
Grindelia squarrosa	18.9±7.6	+
Grindelia stricta subsp. oregana	29.3±5.9	+
Grindelia stricta subsp. venulosa.	25.7±8.2	+
Helianthemum grandiflorum subsp. obscurum	62.3±5.3	++++
Hepatica nobilis	48.6±12.1	++++
Heracleum sphondylium	27.5±6.5	+
Hypericum montanum	21.5±7.2	++++
Hyssopus seravschanicus	25.6±5.2	+
Inula magnifica	46.6±6.2	+++
Jatropha curcas	0.0±0.0	++
Lathyrus pratensis	48.9±7.6	+++
Lathyrus tuberosus	12.8±5.8	+
Lavandula angustifolia	39.9±5.8	++
Lavandula canariensis	18.7±5.2	+++
Lembotropis nigricans	49.7±9.8	+
Leuzea carthamoides	0.0±0.0	+++
Leuzea carthamoides	29.8±5.1	+++
Levisticum officinale	15.8±2.9	+
Lobelia siphilitica	0.0±0.0	+
Lotus corniculatus	18.2± 5.6	++
Lythrum salicaria	100.0±0.0	++++
Lythrum virgatum	100.0±0.0	++++
Medicago falcata	32.8±7.5	+
Melilotus albus	25.6±5.2	++
Mentha arvensis	100.0±0.0	++++
Mentha longifolia	100.0±0.0	++++
Mentha suaveolens	100.0±0.0	++++
Nepeta pannonica	25.5±6.5	+
Ononis arvensis	25.1±5.5	+
Ononis spinosa	3.5±0.9	+++
Onopordon acanthium	16.7±8.2	+
Origanum dictamnus	45.8±7.6	+++
Origanum vulgare	52.6±6.8	++++
Orlaya grandiflora	15.2±3.8	+++
Panax ginseng	22.2±3.6	+
Petasites hybridus	56.7±6.2	+
Phacelia tanacetifolia	62.3±12.1	+++
Physalis alkekengi	45.4±2.9	++
Plantago lanceolata	5.1±2.8	+
Polygonum aviculare	69.2±5.6	+
Populus nigra	56.9±5.3	+++
Potentila argentea	100.0±0.0	++++
Potentilla anserina	32.8±6.9	++++

Table 2. Contd.

Potentilla fruticosa	100.0±0.0	++++
Potentilla hirta	58.9±6.5	++++
Potentilla reptans	45.6±3.9	+++
Pyrethrum parthenium	23.7±8.9	+++
Reynoutria × bohemica	26.7±4.1	+++
Rubia tinctorum	0.0 ±0.0	+
Rumex acetosella	45.5±6.5	++++
Salvia glutinosa	0.0±0.0	+
Salvia officinalis	23.8±7.5	++
Saponaria officinalis	22.7±5.2	+
Scrophularia nodosa	18.5±5.3	++
Securigera varia	20.8±8.6	+
Sedum rosea	28.9±6.3	++++
Senecio umbrosus	15.2±5.9	+++
Seseli pallasii	100.0±0.0	++++
Schisandra chinensis	5.1±1.7	++
Silene vulgaris	32.5±7.2	+
Silphium perfoliatum	3.2±1.8	+
Stachys byzantina	28.9 ±6.3	++
Stachys palustris	38.7±12.5	+
Stachys recta	25.1±6.3	+
Stachys sylvatica	12.5±3.8	+
Teucrium botrys	34.6±5.5	+++
Teucrium capitatum	32.5±6.5	+
Teucrium hircanicum	0.0±0.0	+++
Teucrium chamaedrys	12.8±3.9	+++
Teucrium chamaedrys	100.0±0.0	++++
Thymus alpestris	39.2±5.6	+++
Thymus fragrantissimus	0.0±0.0	++
Thymus serphyllum	53.9±6.7	+++
Trigonella foenum-graecum	87.7±5.9	++
Valeriana officinalis	82.6±5.9	+++
Verbena hirta	0.0±0.0	++
Vincetoxicum hirundinaria	100.0±0.0	++++
Withania somnifera	10.3±2.8	+

* Average mortality (± S.E.) observed on the 5th day, ** Effectiveness of extracts on larval growth inhibition, where; + smaller than 10%, ++ from 10 to 25%, +++ from 25 to 50%; ++++ larger than 50 %.

and *P. argentea*, which caused more than 95% reduction of larval growth. Application of lethal doses did not cause reduced food intake in all the extracts (Table 3). The highest antifeedant effect was found for 4 extracts (*A. chamaepitys*, *A. archangelica*, *F. vulgare* and *V. hirundinaria*) where FDI 99 to 100% was found. FDI 10 to 50% was found for 9 extracts, and almost no significant effect was determined for the extracts from *S. pallasii* and *L. salicaria*.

DISCUSSION

Our study demonstrates the effect of methanol extracts obtained from 134 Eurasian plant species on the mortality and larval growth of *S. littoralis*. The combination of efficiency on mortality and larval growth inhibition was chosen as the main criterion for selecting plants that would be prospective for the development of new botanical insecticides. Based on these criteria, 19 extracts were selected, which caused both 100% larval mortality and growth inhibition higher than 75% after application of 15 mg dose of the extract in 1 g of food. Lethal doses and the effect of LD_{50} on growth inhibition and the antifeedant effect were estimated in order to determine the difference in efficiency of the selected extracts. If mortality was observed as the most important criterion, the extract from *A. archangelica* seeds could be

Table 3. Lethal doses, antifeedant and growth inhibition activity of most effective extracts against larvae of *S. littoralis*.

	LD_{50} (CI_{95})[a] (mg/g)	Chi[b]	FDI[c] (%)	GI[d] (%)
Ailanthus altissima	4.8 (3.8-5.3)	3.882	22.2 ± 3.8^{ef}	96.4± 2.1^{a}
Ajuga chamaepitys	9.9 (8.9-10.3)	2.518	100.0 ± 0.0^{a}	29.5 ± 5.3^{e}
Ajuga reptans	3.7 (3.0-4.4)	0.067	31.5 ± 2.8^{e}	90.1 ± 8.2^{ab}
Angelica archangelica	0.4 (0.3-0.5)	1.033	99.3 ± 1.8^{a}	69.5 ± 3.3^{c}
Artemisia campestris	7.4 (5.5-11.8)	2.057	42.1 ± 5.6^{d}	78.2 ± 5.1^{bc}
Buphtalmum salicifolium	8.7 (6.9-12.9)	0.368	17.8 ± 5.6^{f}	84.7 ± 5.7^{b}
Camellia sinensis	2.6 (1.8-3.3)	0.036	26.7 ± 8.9^{ef}	92.9 ± 3.5^{ab}
Chenopodium bonus-henricus	8.9 (8.1-9.9)	0.192	81.9± 6.7^{b}	48.7 ± 6.2^{de}
Eupatorium cannabinum	10.2 (9.8-11.3)	1.512	64.2 ± 5.9^{c}	31.6 ± 5.4^{e}
Foeniculum vulgare	9.3 (7.9-10.5)	1.333	100.0 ± 0.0^{a}	85.5 ± 3.2^{b}
Lythrum salicaria	2.3 (1.3-2.9)	0.085	-1,7± 5.2^{g}	96.6 ± 5.3^{a}
Lythrum virgatum	6.1 (4.3-8.9)	0.295	23.5 ± 7.6^{ef}	98.4 ± 3.2^{a}
Mentha arvensis	3.5 (3.1-4.8)	2.061	52.5 ± 3.2^{c}	60.7 ± 5.2^{c}
Mentha longifolia	4.5 (3.3-6.5)	0.053	55.1 ± 6.3^{cd}	65.3 ± 3.3^{c}
Mentha suaveolens	7.3 (6.3-8.5)	0.746	27.4± 5.3^{ef}	77.3 ± 7.8^{bc}
Potentila argentea	3.6 (3.0-4.2)	3.957	11.4± 2.8^{f}	95.1 ± 2.8^{a}
Potentilla fruticosa	5.8 (4.3-7.2)	1.065	17.3 ± 5.2^{f}	99.1 ± 3.2^{a}
Seseli pallasii	8.6 (6.9-9.9)	0.700	3,3± 2.8^{g}	56.2 ± 7.3^{cd}
Vincetoxicum hirundinaria	6.0 (4.8-7.8)	1.364	100. 0 ± 0.0^{a}	93.5 ± 5.8^{a}

[a]Lethal doses in mg/cm^3, CI_{95} denotes confidence intervals, compound activity is considered significantly different when the 95% CI fail to overlap. [b]Chi-square value, significant at $p < 0.05$ level. [c] Feeding deterrent index (mean ±S.E.), numbers present the deterrent (positive numbers) and preference (negative numbers) effect after exposure lethal doses of extracts. [d] Growth inhibition (mean ±S.E.) effect after exposure lethal doses of extracts. Mean values followed by same letters in a column are statistically not significant by Tukey's HSD at $p<0.05$.

chosen as the most efficient one for its LD_{50} was significantly lower (0.4 mg/g) compared to the other extracts. However, if we take into account larval growth inhibition, extracts from *L. virgatum*, *L. salicaria*, *A. altissima*, *P. fruticosa* and *P. argentea,* which caused larval growth inhibition by more than 95% (Table 3), could also have been selected. While compounds that cause mortality have an immediate effect on reduction of pest numbers, compounds that can be classified as insect growth regulators and/or inhibitors (IGRs) affect the ability of insects to grow and mature normally. IGRs are sought and developed for their high activity and selectivity against insects with inherently low toxicity to non-target wildlife (Darvas and Polgar, 1998).

As a result of their mode of action, the subtle effect of these compounds is likely to pose a greater effect to immature stages than to adults of a number of insect species (Smagghe et al., 1999). Most compounds that belong to the IGRs class are not stomach or neurotoxic poisons, but have a unique mode of action that disrupts the molting process or cuticle formation in insects (Smagghe and Degheele, 1994) or interferes with the hormonal balance of insects (Céspedes et al., 2000; Pavela et al., 2005). They are characteristically slow acting against a narrow range of sensitive stages of the insects' life cycle with harmful effect against target pests (Casida and Quistad, 1998). However, additional detailed experiments are needed to determine the mechanism of the effects of our extracts on insects, and shed light on these mechanisms. Besides larval growth, inhibition may also be due to reduced food intake caused by the antifeedant effect.

However, our results showed that although extracts from *Lythrum* sp., *A. altissima* and *Potentilla* sp. did exhibit almost 100% inhibition of *S. littoralis* larval growth, the larvae received food contaminated with the extracts relatively well because FDI was lower than 25% (Table 3). This effect leads to the assumption that the extracts contain GIRs. On the contrary, extracts from *F. vulgare* and *V. hirundinaria* showed both high FID (100%) and GI (85 and 93%, respectively), and it can be thus assumed that growth inhibition was caused predominantly by low food intake and subsequent starvation of the larvae. Although the extracts were not analyzed in our study, groups of secondary metabolites that are a subject of medical research are known at least, in respect of the fact that all the plants selected have been used in medicine (Bruneton, 1999). It can be assumed based on available literature that besides some hydrocarbons form parts of essential oils, the extract from *A. archangelica*

seeds also contains numerous coumarins: simple, furanoid and hydroxyisopropylfuranoid, linear and angular (e.g. osthol, aviprin, imperatorin, bergapten, xanthoxin, angelicin, archangelicin) (Zobel and Brown, 1991; Bruneton, 1999; Murphy et al., 2004). Coumarins have been known for their antifeedant activity (Ballesta-Acosta et al., 2008). Vera et al. (2006) found that coumarins applied in the diet of *S. frugiperda* larvae in the dosage 100 µg extended larval duration, inhibited their growth, and although the authors did not find any significant mortality in the course of larval development (0 to 20%), high pupal mortality (50 to 80%) and malformed adults (30 to 100%) were determined; moreover, the authors found a mutual synergistic effect between some coumarins. The synergistic effect of coumarins with other phytochemicals contained in the extract from *A. archangelica* seeds may have caused significant larval mortality in our experiments.

Potentilla sp. as well as *Lithrum* sp. contains high percentages of tannins (10 to 25%), flavonoids, phenolics, sterols and terpenes (Bruneton, 1999). These substances are important enzymatic and metabolic inhibitors. Some of them bind to proteins, acting as precipitating agents for nutritional protein, thereby inhibiting insect digestive enzymes and reducing digestibility (Kubo, 1997; Kubo and Kinst-Hori, 1999; Kubo et al., 2000; Pavela et al., 2005). Nomura and Itioka (2002) studied the efficiency of synthesized tannin on *S. litura* larval growth and survival. They found that the tannin applied as part of diet starting from the dosage of 0.2 mg/g, significantly reduced the number of survivors until adults. Higher dosages, approximately from 2 mg/g, cause mortality also in the course of larval development. The work of these authors also shows that GI effects increase with higher tannin dosage without any significant manifestation of an antifeedant effect. This was confirmed also by our results, and although it is clear that the mixture of various tannins in extracts from *Potentilla* sp and *Lithrum* sp. are different in terms of their molecular structure, it can be assumed that their biological efficiency may be similar (Zucker, 1983).

Most of the selected plants belong to verified medicinal plants (Bruneton, 1999), which justifies also the assumption that potential botanical insecticides would be safe for the health. However, both the issue of formulations of the products and the stability, as well as content of effective compounds must be dealt with subsequently. Last but not least, biological efficiency of the product formulations against target and non-target organisms should be verified.

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