SHORT COMMUNICATION

Potential Insecticidal Activity of Extracts from 18 Species of Medicinal Plants on Larvae of *Spodoptera littoralis*

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Abstract

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Insecticidal activity of methanol extracts from 18 species of medicinal plants was tested on 3rd instar larvae of the Egyptian cottonworm (*Spodoptera littoralis*). All extracts were toxic to larvae. Extracts from *Ocimum basilicum, Origanum majorana*, *Picea excelsa* and *Salvia officinalis* were highly toxic (LC_{50} – 1.7, 3.6, 4.1 and 4.7 μg/ml, respectively). Extracts from *Melilotus officinalis, Pinus silvestris, Taraxacum officinalis* and *Achillea ptarmica* were moderately toxic (LC_{50} – 5.6, 7.3, 8.4 and 9.1μg/ml, respectively). The relative growth rate, consumption of food and conversion efficiency of the digested food were calculated. Clear correlations were found between weight increase, quantity of ingested food, and the quantity of excrements produced during the whole assay period. These results indicate an antifeedant property of the tested extracts.

Keywords: plant extract; medicinal plants; *Spodoptera littoralis*; insecticidal activity

The increasing number of investigations on plant-insect chemical interactions (MILLER & MILLER 1988) in the last few decades unveiled the potential of utilising secondary plant metabolites, or allelochemicals, as pest control agents. The interest in botanical insecticides resulted from the need to provide alternatives in IPM programs for the synthetic insecticides whose adverse effects on agro-ecological systems are well known.

The majority of botanicals that were developed as commercial pesticides originated from tropical and subtropical sources. Because of the intensity of plant-insect interactions in tropical and subtropical regions, the plants there have well-developed defence mechanisms against pests and are excellent sources of new insecticidal substances (PRAKASH & RAO 2000). However, plants from the moderate climates of Europe also have secondary metabolites that are an important part of the defense barriers against insects (CHERMENSKAYA & BUROV 1998). For example, medicinal plants can and have been screened for anti-insect substances (PASCUAL-VILLALOBOS & ROBLEDO 1998; CICCIA *et al*. 2000; PAVELA 2002). Sampling the native flora might also lead to the discovery of new compounds. Several species of the family *Lamiaceae* contain biologically

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active substances that are used in medicine. These substances belong to the group of phenolic acids, mono- and di-terpenes, flavonoids and limonoids (Bruneton 1999) which could also be responsible for insecticidal activity.

In this paper, we present the results of testing the potential insecticidal activity of extracts from 18 medicinal plants. As a model system we chose an important polyphagous pest, the Egyptian cotton leaf worm *Spodoptera littoralis* Boisdual (*Lepidoptera*: *Noctuidae*).

MATERIAL AND METHODS

Tested plant material. The 18 plant species and their parts used are listed in Table 1. Methanol extracts were prepared by standard procedures commonly used to screen plant extracts with insecticidal potential (Stein & Klingauf 1990).

The air-dried plant samples (250 g) were pulverised and the powder was successively extracted by maceration (48 h) in 2500 ml of methanol (96%). The solution was filtered and the filtrate concentrated

Table 1. Botanical data of the plants, & mass yield of extracts (from 250 g of dry matter) tested for toxicity against *Spodoptera littoralis*

Species	Family	Plant stage	Plant material	Collection site	Mass yield (g)
Achillea ptarmica L.	*Asteraceae*	flowering	leaves & flowers	(Leningradsky region) Russia	97.00
Ambrosia artenisiifolia L.	*Asteraceae*	flowering	leaves & flowers	(Leningradsky region) Russia	40.00
Artemisia vulgaris L.	*Asteraceae*	pre-flowering	leaves & buds	Pyatigorsk, Caucasus, Russia	30.25
Cnicus benedictus L.	*Lamiaceae*	pre-flowering	aerial parts	Prague, Czech Republic	35.95
Hyssopus officinalis L.	*Lamiaceae*	flowering	aerial parts	Prague, Czech Republic	50.54
Marrubium vulgare L.	*Lamiaceae*	flowering	leaves	Prague, Czech Republic	68.33
Melilotus officinalis (L.) LAM.	*Fabaceae*	flowering	leaves & flowers	(Leningradsky region) Russia	20.95
Melissa officinalis L.	*Lamiaceae*	flowering	leaves & flowers	Prague, Czech Republic	69.33
Mentha virescence L.	*Lamiaceae*	flowering	leaves & flowers	(Leningradsky region) Russia	62.75
Ocimum basilicum L.	*Lamiaceae*	flowering	leaves & flowers	Prague, Czech Republic	65.82
Origanum majorana L.	*Lamiaceae*	vegetative	aerial parts	Prague, Czech Republic	42.36
Picea excelsa L.	*Pinaceae*	vegetative	needles & branches	Pushkin, St. Petersburg, Russia	35.75
Pinus silvestris L.	*Pinaceae*	vegetative	needles & branches	Pushkin, St. Petersburg, Russia	25.75
Salvia officinalis L.	*Lamiaceae*	flowering	leaves & flowers	Prague, Czech Republic	48.23
Salvia splendens SELLOW	*Lamiaceae*	flowering	leaves & flowers	Prague, Czech Republic	35.32
Sanguisorba officinalis L.	*Rosaceae*	flowering	leaves & flowers	(Leningradsky region) Russia	58.50
Taraxacum officinale Weber	*Asteraceae*	flowering	leaves & flowers	Pushkin, St. Petersburg, Russia	32.25
Veronica officinalis L.	*Scrophulariaceae*	flowering	leaves & flowers	(Leningradsky region) Russia	42.75

under reduced pressure on a rotary evaporator at 30°C. The yields of the extract are presented in Table 1. All extracts were stored at 5°C until used. The dry residue was re-suspended in water to make 10% stock solutions (w/v) that were stored at 5°C for later use.

Insect used. The experiments were performed with 3rd instar larvae (10–12 mg) of *Spodoptera littoralis*, reared on an artificial diet (Premix, prepared by Stonefly Industries, Inc., Bryan, TX, USA) for more than 30 generations. The larvae were kept under L16:D8 photoperiodic regime at a constant temperature of 25 ± 1°C and 70% r.h.

Studied activity. The stock solutions of crude extracts were diluted with water for final concentrations of 0.5, 1.0 and 5.0% (all w/v of the crude extract); 75 ml of these dilutions and of the 10% stock solution (w/v) were stirred into 25 g of the diet mixture (Premix). The diet without an extract but with water added was used as a control. Portions of 100 g diet containing 0, 1250, 2500, 12 500 and 25 000 ppm of chemicals were thus obtained.

Experiment 1 – larval mortality: Third instar larvae were placed on a slice of the diet in Petri-dishes (diameter 15 cm). Five concentrations were tested: 0 (control), 0.5, 1.0, 5.0 and 10.0% (all w/v). The diet was changed every 2 d, surviving larvae received the diet until the pupal stage, and larval mortality was recorded. Each experiment was performed on 25 larvae in 5 replications (n = 125).

Experiment 2 – growth index: Third instar larvae were placed on a slice of the diet in Petri-dishes (diameter 15 cm). They were reared on the diet containing 0.5% (w/v) of the test extracts for 7 d. Each treatment included 5 larvae in 10 replications (n = 50).

The surviving larvae were kept on the diet until the pupal stage when the surviving insects, remaining diet and the produced excrements were weighed, and the rate of mortality was calculated.

The criteria for evaluation of the insecticidal activity were as follows:

(1) Larval mortality – expressed as lethal concentration – LC_{50}

(2) Relative growth rate (RGR) was calculated as: **RGR = (final Wt – initial Wt)/7** and expressed in mg/mg mean Wt per day (Miller & Miller 1988)

(3) Consumption rate (CI) was calculated as **CI = F/T,** where: F is the ingested food during the time interval T (Klein & Kogan 1974)

(4) Efficiency of conversion of the digested food (ECD) was calculated as **ECD = [B/(F – E)] × 100,** expressed in percentages, where B is the biomass gained, F is the ingested food, and E is the excreta (Miller & Miller 1988).

Data analyses and statistics: The RGR and CI data were subjected to analysis of variance (ANOVA, 20). The data for ECD were transformed to arcsine square root [arcsine (percent mortality/100)^2] before statistical analysis. Differences between the treatments were determined by Tukey's test. Differences between means were considered significant at $P \leq 0.05$.

Probit analysis was used to determine LC_{50} and the corresponding 95% confidence intervals.

RESULTS

Each of the plant extracts was more or less toxic to the larvae of *Spodoptera littoralis*, although there were some differences between extracts (Table 2). Four of the extracts were highly toxic to the larvae, with LC_{50} of ≤ 5.0 µg/ml; they were prepared from *Ocimum basilicum* L., *Origanum majorana* L., *Picea excelsa* L. and *Salvia officinalis* L. (1.7, 3.6, 4.1 and 4.7 µg/ml, respectively). Four extracts were moderately toxic, with an LC_{50} from 5.1 to 10 µg/ml; they came from *Melilotus officinalis, Pinus silvestris, Taraxacum officinalis* and *Achillea ptarmica* (5.6, 7.3, 8.4 and 9.1 µg/ml, respectively).

The indexes of RGR, CI and ECD (Table 2) showed clear relationships between weight increase, quantity of diet ingested, and the quantity of excrements produced during the whole assay period. The relative growth rate (RGR) decreased significantly ($P \leq 0.05$) after application of all extracts, except of the extracts from *Cnicus benedictus* and *Mentha virescence*. For the extracts from *Origanum majorana, Ocimum basilicum, Salvia officinalis, Picea excelsa* and *Pinus silvestris* the values of RGR were 3.28, 3.75, 3.76, 3.36 and 3.33 mg/day, respectively, which is 80% lower than in the control group (17.45 mg/day).

Such significant decreases of growth rate correspond with the index of consumption rate (CI) and the efficiency of conversion of digested food (ECD) (Table 2). Consumption of the diet treated with extracts from *Pinus silvestris, Melilotus officinalis, Origanum majorana* and *Ocimum basilicum* was decreased by 60% in comparison with the control. Similarly, significant decreases were observed for the efficiency of conversion of digested food. Extracts of *Origanum majorana, Ocimum basilicum,*

Table 2. The effect of plant extracts (0.5%, w/v) on relative growth rate (RGR), consumption rate (CI), efficiency of conversion of digested food (ECD) by and lethal concentration (LC_{50}) for larvae of *Spodoptera littoralis*

Species	*RGR (mg/day) (± S.E.)	*CI (mg/day) (± S.E.)	*ECD (%) (± S.E.)	**LC_{50} (μg/ml) (95% ci)
Achillea ptarmica	5.22 (1.07)bc	20.97 (1.26)b	25.4 (2.3)bc	9.1 (8.2–10.1)
Ambrosia artenisifolia	4.26 (0.66) bc	17.59 (0.99)bc	40.8 (2.1)b	11.6 (10.9–11.9)
Artemisia vulgaris	9.16 (1.15)b	15.28 (1.67)bc	32.1 (3.6)bc	10.7 (10.1–11.2)
Cnicus benedictus	12.93 (2.94)ab	16.91 (0.76)bc	31.6 (5.2)bc	137.9 (120.0–148.0)
Hyssopus officinalis	5.47 (0.33)c	21.93 (0.97)b	25.5 (5.2)bc	17.8 (16.0–18.8)
Marrubium vulgare	8.26 (0.35)b	16.33 (1.19)bc	20.1 (4.3)c	53.8 (49.2–55.6)
Melilotus officinalis	4.56 (0.96) c	12.96 (1.26)c	21.6 (1.1)c	5.6 (5.3–5.9)
Melissa officinalis	4.24 (0.98)c	16.10 (1.38)bc	26.3 (7.1)bc	37.4 (32.1–42.3)
Mentha virescence	12.53 (1.12) ab	16.33 (1.33)bc	40.2 (1.2)b	36.9 (33.3–42.1)
Ocimum basilicum	3.75 (0.67) c	12.66 (0.58)c	26.3 (4.1)bc	1.7 (1.5–1.9)
Origanum majorana	3.28 (0.92)c	13.62 (1.57)c	19.2 (5.5)c	3.6 (3.3–3.9)
Picea excelsa	3.36 (0.63)c	12.96 (1.56)c	20.2 (2.2)c	4.1 (3.3–5.1)
Pinus silvestris	3.33 (0.26)c	13.79 (0.96)c	37.2 (2.3)b	7.3 (6.9–7.6)
Salvia officinalis	3.76 (1.52)c	17.45 (1.33)bc	22.2 (8.1)bc	4.7 (4.1-5.8)
Salvia splendens	5.23 (0.99)c	18.61 (1.33)bc	30.1 (3.5)b	77.1 (60.0–82.1)
Sanguisorba officinalis	8.12 (1.03)b	15.61 (1.03)bc	29.2 (1.5)bc	18.1 (15.3–20.3)
Taraxacum officinale	5.35 (0.69) bc	16.42 (1.33)bc	33.2 (1.5)bc	8.4 (8.0–8.8)
Veronica officinalis	6.22 (0.22)bc	16.47 (1.21)bc	25.6 (2.1)bc	16.7 (15.2–18.3)
Control	17.45 (3.28)a	32.9 (1.46)a	54.5 (3.3)a	

Average (± S.E.), 95% ci denotes confidence interval; *n = 50, **n = 150
Figures followed by different letters are significantly different at $P \leq 0.05$ (Tukey)
RGR = relative growth rate; CI = consumption rate; ECD = efficiency of conversion of digested food

Marrubium vulgare, Hyssopus officinalis, Salvia officinalis, S. splendens, Mellilotus officinalis, Sanguisorba officinalis, Picea excelsa, Achillea ptarmica, Veronica officinalis and *Melissa officinalis* lowered the conversion of digested food by 45 to 65% in comparison with the control.

DISCUSSION

They results suggest that the extracts had a strong antifeedant (Schmutterer 1995) as well as toxic effect, as the toxins can cumulate in the body of larvae and subsequently cause mortality (Miller & Miller 1988). From this type of study it is not possible to discern if both of these are occurring. Nutritional experiments that can differentiate behavioural effects and post-ingestive toxicity have been conducted and the results reported elsewhere. They results show that the extracts act mainly as antifeedants, with some chronic toxicity. Some extracts may have a contact action, via substances such as monoterpenoids, on the nervous system of larvae (Isman 2000).

Of the plants used in our experiment, species from the families *Lamiaceae* and *Pinaceae* (syn. *Abiateae)* had the highest biological activity. These strongly aromatic plants contain a number of biologically active substances that are often used in pharmacology (Bruneton 1999) or as spices. Their aromatic substances belong particularly to the monoterpenoids and are part of the essential oils extracted from these plants.

Recent studies have also indicated efficacy of essential oils against pests on plants. Essential oils of cumin (*Cuminum cyminum*), anise (*Pimpinella anisum)*, oregano (*Origanum syriacum* var. *bevanii)* and eucalyptus (*Eucalyptus camaldulensis*) were effective as fumigants against the cotton aphid (*Aphis gossypii*) and the carmine spider mite (*Tetranychus cinnabarinus*) – two greenhouse pests (Tuni & Sahinkaya 1998). The efficacy of basil (*Ocimum* spp.) against garden pests has recently been reviewed (Quarles 1999). Lee *et al*. (1997) reported the toxicity of a range of essential oil constituents to the western corn rootworm (*Diabrotica virgifera*), the two-spotted spider mite (*Tetranychus urticae*) and the housefly (*Musca domestica*). Dietary effects of a number of monoterpenoids against the European corn borer (*Ostrinia nubilalis*) have been recently reported (Lee *et al*. 1999). There is also evidence that certain essential oils and their constituents are effective against *Varroa jacobsoni*, an ectoparasite of the honey bee (Calderone *et al*. 1997).

Only a few plant species included in our study have been reported to be active by other authors (Prakash & Rao 2000). However, the studies which are comparing the effects of extracts from more species of medicinal plants of Euro-Asiatic areas on mortality and larval growth were not systematically elaborated until this time.

Species of the families *Lamiaceae*, *Asteraceae* and *Pinaceae* (syn. *Abiateae*) were reported to show antimicrobial activity (Bruneton 1999). It was also found that *Origanum* spp., *Salvia* spp., *Pinus* spp. and *Artemisia* spp. had pesticidal properties (Prakash & Rao 2000). A number of these species or close relatives have been studied for pharmaceutical purposes but the compounds isolated from them need not be responsible for the insecticidal activity. For example, the repellency shown by *Artemisia barrelieri* extracts to *Tribolium castaneum* (Herbst. 1797) larvae may be in our experiment unrelated to artemisin in *Artemisia annua*, a compound with antimalarial activity (Charles & Simon 1990). Our contribution in this case is either to identify more promising species of the same genera or new activities.

Only one insect species was used in our tests and, therefore, the activity reported is restricted only to the described conditions. In addition, the species of plants used represent only a small sample of all species present in the Euro-Asian region. To identify an active compound is a difficult task, and so is the testing of its biological activity on insects because of the large variability in their responses.

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Souhrn

Pavela R., Chermenskaya T. (2004): **Potenciální insekticidní účinky extraktů získaných z 18 druhů léčivých rostlin proti housenkám** *Spodoptera littoralis.* Plant Protect. Sci., **40**: 145–150.

Insekticidní účinky metylalkoholových výtažků z 18 druhů léčivých rostlin byly testované na larvách třetího instaru blýskavky (*Spodoptera littoralis*). Všechny výtažky vykázaly insekticidní aktivitu. Nicméně, byly zjištěné rozdíly mezi jednotlivými druhy rostlin. Výtažky z *Ocimum basilicu*m, *Origanum majorana*, *Picea exelsa* a *Salvia officinalis* byly vysoce jedovaté (LC_{50} 1,7; 3,6; 4,1 a 4,7 µg/ml). Výtažky z *Melilotus officinali*s, *Pinus silvestris*, *Taraxacum officinalis* a *Achillea ptarmica* byly středně jedovaté (LC_{50} 5,6; 7,3; 8,4 a 9,1 µg/ml). Pro všechny výtažky byly zjištěny indexy; index relativního růstu larev, indexy spotřeby a využití potravy a indexy využitelnosti potravy k růstu larvami *S. littorali*s. Výsledky ukazují na převážně antifidantní účinky většiny testovaných extraktů.

Klíčová slova: rostlinné extrakty; léčivé rostliny; *Spodoptera littoralis*; insekticidní účinky

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