Effectiveness of Some Botanical Insecticides against *Spodoptera littoralis* Boisduvala (Lepidoptera: Noctudiae), *Myzus persicae* Sulzer (Hemiptera: Aphididae) and *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Abstract

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Abstract: Biological efficiency of botanical insecticides was determined that were obtained from *Pongamia glabra*, *Azadirachta indica* and *Chrysanthemum cinerariifolium* against *Spodoptera littoralis*, *Myzus persicae* and *Tetranychus urticae* on greenhouse plants. In all the tested extracts, the highest concentration caused 100% mortality. In the other tested concentrations, a conclusive difference in efficiency was found; on day 12 after application, the highest efficiency was determined for *M. persicae* pongam oil, for *T. urticae* and *S. littoralis* neem oil.

Keywords: *Spodoptera littoralis*; *Myzus persicae*; *Tetranychus urticae*; botanical insecticides; *Azadirachta indica*; *Chrysanthemum cinerariifolium*; *Pongamia glabra*

Botanical pesticides are an important group of naturally occurring, often slow-acting crop protectants that are usually safer to humans and the environment than conventional pesticides, and with minimal residual effects. Moreover, thanks to the fact that botanical pesticides contain mixtures of biologically active substances, no resistance is developed in pests and pathogens. Therefore the use of plant pesticides has been recommended ever more as a suitable alternative of plant protection with minimum negative risks (Isman 2006; Pavela 2007). Especially botanical insecticides have long been a subject of research in an effort to develop alternatives to conventional insecticides. The use of plant insecticides has a long-term tradition in Europe; the first known written references to the application of plant extracts against pests come from Rome and date back to about 400 B.C. (Dayan 2009). At present, several dozens of plant insecticide are used worldwide, based on various extracts, especially of the families Rutaceae, Lamiaceae, Meliaceae and Asteraceae.

Although plant pesticides have been studied in many laboratory tests (Chandler 1951; Morgan 2009), very few studies are available that present results from practical use, and there is a great lack of biological efficiency comparisons of several products on multiple pest species at the same time. In our study, we therefore present the results of biological efficiency of two new and one

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commercial formulation of plant insecticides. The products were tested in three different dosages for mortality of 3 pest species. The products were based on different efficient substances.

The first product was prepared as emulsified oil from the seeds of *Azadirachta indica* A. Juss. Neem seed extracts rich in azadirachtin (10–25%) act both as potent antifeedants and as insect growth regulators (Prakash & Rao 1986; Koul 1992; Morgan 2009). The role of other triterpenoids (nimbin, salanin, and derivatives thereof) present in neem seed extracts, as contributors to overall bioactivity, is controversial and most evidence points to azadirachtin as the most important active principle (Isman 1996; Pavela *et al*. 2004).

The second product was developed from oil obtained from the seeds of *Pongamia pinnata* Pierre. *P. pinnata* is one of the non-edible oil yielding leguminous tree species of Indian origin. Seeds of *P. pinnata* contain bitter, red brown, thick non-drying, non-edible oil (27–39%), used for tanning leather, soap making, to treat various ailments (Meera *et al*. 2003) and as an illuminating oil in the rural areas of India. Antibacterial activity of the oil was shown against *Bacillus*, *E. coli*, *Pseudomonas*, *Salmonella*, *Staphylococcus* and *Xanthomonas* (Kumar & Kalidhar 2003). Seed oil contains two flavonoids, Pongamol and Karanjin, which makes it unsuitable for edible purposes. Pongam oil has been recognised as "Biodiesel", as several parameters of diesel and *P. pinnata* oil are comparable (Parmar *et al*. 1976).

The commercial product based on pyrethrin was selected as the standard product. Pyrethrin is a natural botanical insecticide extracted from the flower petals of *Chrysanthemum cinerariifolium* (Trev.) Vis. The active insecticidal ingredients of pyrethrin are the esters pyrethrin I and II, cinerin I and II, and jasmolin I and II (Chandler 1951). These effective compounds have high lethal activity toward insects, low mammalian toxicity, and short environmental lifetimes (Henry *et al*. 1999). Due to these useful characteristics, pyrethrin liquid and aerosol formulations are applied as insecticides in households, restaurants and food storage areas and on many crops. In addition, pyrethrin is used as an all-natural, organic insecticide for the control of human lice and mosquitoes (Streit 1994).

In our study, three representatives of polyphagous pests were selected to determine biological efficiency: *Spodoptera littoralis* Boisduvala, *Myzus persicae* Sulzer, and *Tetranychus urticae* Koch.

MATERIAL AND METHODS

Chemicals. **Pyrethrum** – Spruzit® Schädlingsfrei (W. Neudorff GmbH KG, Germany) 25% emulsion of *C. cinerariifolium* extract in rapeseed oil, which corresponds to the content of a.i. 4.59 g/l Pyrethrins. **Neem** – seed oil from *Azadirachta indica* (Parker Group, India) emulsified using Tween 85. The oil was tested by High Performance Liquid Chromatography (HPLC) to determine the azadirachtin, nimbin and salanin content that is over 2000, 4000, and 6000 ppm, respectively. **Pongam** – seed oil from *Pongamia pinnata* (Parker Group, India) emulsified using Tween 85. The oil was tested by HPLC to determine the Karanjan content that is over 22 000 ppm.

Plants. Tomato (*Lycopersicon esculentum* Miller) and cucumber (*Cucumis sativus* L.) plants were used as experimental plants, planted in beds in the greenhouse, at the spacing of 80 × 100 cm. At the time of the experiment, the plants were 80–100 cm high and were found in the flowering phase and formation of the first small fruits. The plants were placed in a greenhouse with controlled moderate temperature; the temperature was in the range of 21–28°C and 16:8 (L:D) photoperiods were maintained during the experiment.

Bioassays. All tested insects, Egyptian cotton leafworm, *Spodoptera littoralis* Boisduvala (Lepidoptera: Noctudiae), Green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), and Spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) were obtained from RCI breeds (laboratory colony). Before establishing the experiments, a sufficient number of larvae and/or adult individuals were introduced onto the plants in the following manner.

Aphids and spider mites. A sufficient number of nymphs and adult individuals of *T. urticae* or *M. persicae* as appropriate were transferred onto the cucumber or tomato plants using a small brush. Approximately after 5 days upon introduction, the number of live nymphs and adult individuals was determined on marked leaves, on marked shoots, using a portable binocular. The average number of *T. urticae* or *M. persicae* larvae and nymphs from 5 plants was considered to represent the initial number of pests in the efficiency calculation.

Caterpillars: Bioassays were conducted using larvae (weight 25–30 mg) of the tobacco cutworm, *S. littoralis*, obtained from an established laboratory colony (> 50 generations; out-crossed once). Insects were reared on an artificial diet (Instant soybean-wheat germ insect diets, Stonefly Industries, Bryan, TX, USA).

Fifty larvae were always introduced onto tomato leaves, while placing 10 larvae on each leaf. The leaves were spaced randomly among the tomato plants. The number of 50 larvae was considered to represent the initial number of pests in the efficiency calculation.

Application. The field experiments were carried out during the growing seasons of 2007 and 2008 in a greenhouse of the Crop Research Institute, Prague, Czech Republic.

Pongam, Neem and Pyrethrum at rates of 3.0, 1.0 and 0.5% water solutions and untreated plants were used. The size of the plot for tested plants was 25 m^2. Four replications were arranged in a randomised block design. All treatments were applied at 3.4 bars (0.34 MPa) with 500 l/ha, using a SOLO 432 backpack sprayer with a solid cone swirl nozzle.

Mature and immature stages of pests were recorded as follows: 24 days before and 2, 7, and 12 days after treatment.

Statistics. Biological efficacy was determined as percentage mortality on the 2^{nd}, 7^{th}, and 12^{th} day after the application of botanical insecticides. Data from the insecticide trials were analyzed according to the study and sample date, with percentage data being subjected to arc-sine square-root transformation before analysis. All data were analysed by the analysis variance (ANOVA) for a randomised complete block design. Means were separated using Tukey's honestly significant difference (HSD) multiple comparisons test ($P < 0.05$).

Feeding assay with leaf disks.The antifeedant activity of the extracts against the 4^{th} instar larvae of *S. littoralis* was investigated by a no-choice test using leaf disks. Test solutions were prepared from the formulations by further dilution in acetone to produce four different concentrations: 0.05%, 0.5%, 1.0%, and 3%. Leaf disks (28 mm in diameter) were prepared from tomato leaves using a cork borer and weighed before the test. Each disk was dipped in the test solution for 10 seconds. Control leaf disks were dipped in acetone for the same period of time. All disks were left at room temperature for 5 min to evaporate the solvent. In the no-choice test, each arena contained only one treated leaf disk (n = 20 for each treatment). Meanwhile, a group of 20 arenas with one larva and one control disk in each was set up for control. After 4 h, the remnants of leaf disks were collected and dried separately at 60°C to a constant weight. The amount of consumed food was calculated depending on the initial fresh weight of each disk and the dry weight of its remnants, using a standard curve of the relation between fresh weight and dry weight of different sized leaf pieces. The antifeedant index (AFI) was calculated from the formula:

$$AFI = [(C - T)/(C + T)] \times 100 \text{ (in \%)}$$

where:
C – consumption of control disks
T – consumption of treated disks (Pavela *et al.* 2008)

In the no-choice tests, the food consumed by 20 animals that were given control disks was averaged, and the mean was used as C for the calculations of the AFI for each observed T. The experiment was carried out at 25 ± 1°C, RH 65 ± 5% and light regime. The antifeedant indexes at different treatments were compared using the analysis of variance (ANOVA) followed by Tukey's test ($P < 0.05$) for multiple comparisons where significant differences were observed.

RESULTS

All tested botanical insecticides (BI) showed high efficiency in mortality of the tested pests. In spite of that, significant differences in efficiency were found.

The efficiency of BI against aphids is shown in Table 1. In all the tested extracts, the highest concentration caused 100% mortality of aphids on day 12 after application. In the other tested concentrations, a conclusive difference in efficiency was found; on day 12 after application, the highest efficiency was determined in pongam oil ranging (depending on the years) from 96% to 97% and 76% to 82% for 1.0% and 0.5%, respectively. Neem in the concentration of 0.5% showed the lowest efficiency, which was estimated to be approximately 57%. While in pongam oil the efficiency was rising in time, in neem and pyrethrum the efficiency was decreasing slightly, which was caused by the hatching of new nymphs.

The acaricide effect is shown in Table 2. The product based on neem and pongam oil showed to be the most efficient against *T. urticae*. Mortality was rising in time up to 100% for 3% as well as 1% concentration. For the lowest tested concentration, significantly better efficiency was found in neem (60–70%) compared to pongam (49–52%). The product based on pyrethrum was the least

Table 1. Effect of botanical insecticides against *Myzus persicae*

Treatment	Concentration (%)	*n* (before application)		Biological efficacy (%)					
				2 days		7 days		12 days	
		2007	2008	2007	2008	2007	2008	2007	2008
Neem	3.0	120*	168*	97.2^{ab}	95.8^{a}	98.5^{ab}	91.5^{b}	100.0^{a}	100.0^{a}
	1.0	126	186	92.3^{ab}	89.6^{b}	85.3^{b}	80.3^{c}	79.5^{bc}	72.3^{bc}
	0.5	138	169	63.5^{c}	71.3^{c}	58.6^{c}	59.6^{d}	57.8^{d}	56.3^{d}
Pyrethrum	3.0	112	192	100.0^{a}	98.3^{a}	100.0^{a}	99.8^{a}	100.0^{a}	100.0^{a}
	1.0	132	203	99.2^{b}	95.6^{a}	98.5^{ab}	92.1^{ab}	89.5^{b}	78.8^{bc}
	0.5	162	168	98.3^{ab}	89.5^{ab}	96.3^{b}	85.3^{bc}	90.1^{b}	68.5^{c}
Pongam	3.0	128	178	97.5^{ab}	92.7^{ab}	98.1^{b}	99.6^{a}	100.0^{a}	100.0^{a}
	1.0	132	165	89.2^{b}	82.3^{b}	92.3^{b}	90.8^{b}	96.2^{ab}	97.3^{ab}
	0.5	133	155	70.1^{bc}	76.8^{bc}	69.4^{c}	73.5^{c}	76.8^{c}	82.9^{b}
Control		124	198	132**	209	156	238	196	263

*Mean number of aphids before application and **for control plants in the course of experiment

Means followed in the same column by the same letter are not significantly different ($P \leq 0.05$; Tukey's HSD test)

efficient, and the efficiency of only 10–20% was found at 0.5% concentration.

The efficiency against *S. littoralis* larvae is shown in Table 3. No significant difference in efficiency against mortality was found between pyrethrum-based and neem-based BI among the particular concentrations, although the product based on neem showed to cause higher mortality. The lowest efficiency was shown by the product based on pongam oil, as the larval mortality did not even reach 50% for the 0.5% concentration in both years.

Table 2. Effect of botanical insecticides against *Tetranychus urticae*

Treatment	Concentration (%)	*n* (before application)		Biological efficacy (%)					
				2 days		7 days		12 days	
		2007	2008	2007	2008	2007	2008	2007	2008
Neem	3.0	45*	60*	99.3^{a}	98.7^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
	1.0	52	78	98.3^{a}	96.5^{a}	97.2^{a}	91.5^{b}	100.0^{a}	100.0^{a}
	0.5	60	81	75.5^{c}	72.1^{c}	87.3^{bc}	85.6^{c}	60.5^{c}	70.8^{c}
Pyrethrum	3.0	48	80	97.1^{a}	95.6^{a}	93.2^{ab}	90.6^{bc}	98.5^{a}	93.8^{b}
	1.0	50	72	89.2^{ab}	86.3^{b}	92.1^{ab}	89.6^{bc}	60.1^{c}	56.3^{d}
	0.5	53	86	76.5^{bc}	70.2^{c}	76.5^{c}	72.3^{d}	20.1e	10.2^{e}
Pongam	3.0	58	75	98.1^{a}	91.5^{ab}	100.0^{a}	99.2^{a}	100.0^{a}	100.0^{a}
	1.0	52	78	96.3^{a}	89.2^{b}	97.8^{a}	92.8^{b}	82.6^{b}	100.0^{a}
	0.5	63	82	87.6^{b}	82.3^{bc}	92.1^{b}	90.6^{b}	48.9^{d}	52.6^{d}
Control		60	69	57**	49	68	57	76	69

*Mean number of *T. urticae* before application and **for control plants in the course of experiment

Means followed in the same column by the same letter are not significantly different ($P \leq 0.05$; Tukey's HSD test)

Table 3. Effect of botanical insecticides against *Spodoptera littoralis*

Treatment	Concentration (%)	*n* (before application)		Biological efficacy (%)					
				2 days		7 days		12 days	
		2007	2008	2007	2008	2007	2008	2007	2008
Neem	3.0	50*	50*	24.2^{cd}	35.8^{b}	48.8^{cd}	62.6^{c}	98.6a	100.0^{a}
	1.0	50	50	26.6^{c}	29.1^{c}	47.6^{cd}	53.2^{cd}	86.5^{ab}	98.2^{ab}
	0.5	50	50	12.3^{d}	12.6^{d}	29.3^{a}	36.8^{d}	68.5^{b}	72.3^{bc}
Pyrethrum	3.0	50	50	86.5^{a}	78.2^{a}	100.0^{a}	96.7^{a}	100.0^{a}	98.3^{a}
	1.0	50	50	82.3^{a}	72.1^{a}	87.5^{b}	82.3^{b}	88.3^{b}	88.6^{b}
	0.5	50	50	58.3^{b}	44.6^{b}	63.5^{c}	52.1^{cd}	66.8^{b}	62.7^{c}
Pongam	3.0	50	50	82.7^{a}	85.3^{a}	96.5^{a}	92.5^{a}	97.3^{a}	98.8^{a}
	1.0	50	50	30.1^{c}	36.5^{b}	46.5^{cd}	49.8^{cd}	56.8^{c}	62.5^{c}
	0.5	50	50	29.3^{c}	31.8^{bc}	41.8^{d}	36.5^{d}	47.5^{c}	49.2^{c}
Control		50	50	50***	50	48	46	47	43

*Mean number of *S. littoralis* before application and ***for control plants in the course of experiment

Means followed in the same column by the same letter are not significantly different ($P \leq 0.05$; Tukey's HSD test

Antifeedant activity was determined in all extracts in a short-term no-choice test (Table 4). Nevertheless, the neem-based product led to the highest inhibition of food intake, with the antifeedant index equal to 100% for 3–0.5% concentration and 92% for 0.05% concentration. The pyrethrum-based product showed the lowest inhibition of food intake, with the antifeedant index estimated as 31% for the highest tested concentration.

DISCUSSION

In general, botanical insecticides are considered (with some exceptions) as plant protection, which is environmentally safe and harmless to health. In the world, BI are often applied where the production of safe foodstuffs is expected. Safe foodstuffs are produced especially by ecological farms. Besides ecological producers, plant insecticides have been used ever more by vegetable producers when other BI cannot be used (due to protective times) to provide protection against common greenhouse pests, such as aphids, spider mites or caterpillars of phytophagous Lepidoptera (Isman 2005, 2006; Pavela 2007).

Table 4. The antifeedant activity (in %) of botanical insecticides against the 4th instar larvae of *S. littoralis*

Concentration (%)	Pyrethrum	Neem	Pongam
3.00	31.0 ± 13.9^{Ab}	100.0 ± 0.0^{Ba}	99.7 ± 0.5^{Bb}
1.00	46.0 ± 10.8^{Ab}	100.0 ± 0.0^{Ba}	99.2 ± 0.9^{Bb}
0.50	31.1 ± 14.6^{Ab}	100.0 ± 0.0^{Ca}	85.3 ± 13.6^{Bb}
0.05	14.4 ± 6.9^{Aa}	92.1 ± 6.4^{Ca}	45.8 ± 25.1^{Ba}

Means followed in the same column (small letters) or in the same line (caps) by the same letter are not significantly different ($P \leq 0.05$; Tukey's HSD test)

In our study, we showed that single application of a relatively high dosage of 3% solution caused up to 100% mortality of all tested pests. Nevertheless, this dosage is too high from the economic point of view, and is not used vary much in practical application. We chose this concentration as the highest limit dosage, especially in order to observe possible phytotoxicity. Nevertheless, no symptoms of phytotoxicity were found in the tested plants during the experiments. In practical application, the most commonly used concentration for formulations of plant insecticides with the contact effect is 0.5–1% (or 1–3 l/ha in the corresponding dosage). In our experiments, the concentration of 0.5% and 1% showed to be sufficient to provide a significant reduction in the number of pests, and upon repeating the spraying application in the commonly recommended interval of 7–10 days after the first application; it is likely that sufficient protection would be achieved.

BI based on extracts from *A. indica* and *P. pinnata* seeds showed to be a significantly better acaricide compared to the product based on *C. cinerariifolium* extract. This may have been caused by the biological activity of active substances. The active ingredients in pyrethrum extract consisting of a mixture of pyrethrin I (40%), pyrethrin II (36%), cinerin I, and cinerin II (12%) are obtained from dried flowers of the pyrethrum daisy. Technical grade pyrethrum, the resin used in formulating commercial insecticides, typically contains 20–25% of pyrethrins (Dayan *et al.* 2009). Pyrethrins I and II account for a major part of the insecticidal activity, and have been used as insecticides since ancient times. Initial effects include paralysis followed by death. Most flying insects are highly susceptible to pyrethrins, causing them to 'drop' almost immediately upon exposure whereas hyperactivity and convulsions are common in most insects. The mode of action of pyrethrins relates to their ability to affect the sodium channel function in the neuronal membranes. Natural pyrethrins are unstable in light compared with synthetic derivatives (pyrethroids). Pyrethrum is the predominant botanical in use, accounting for 80% of the world botanical insecticide market (Isman 2005). Thus, the contact effect may be insufficient in pests that reproduce by eggs (for example *Tetranychus urticae, Trialeurodes vaporariorum*).

While in pyrethrum no ovicide, antifeedant and growth-inhibiting effects were found, such effects that increase the persistence of the product have been known in BI based on *A. indica* and *P. pinnata* extracts. Effects of neem extracts have probably been explained best, currently considered as one of the most efficient botanical insecticides. These BI, which contain biologically active substances from the limonoid group (azadirachtin, salanin, nimbilin ect.), show significant insecticide, growth-inhibiting and antifeedant effects (Isman 1994; Morgan 2009). While many sources concerning the insecticide activity of azadirachtin and neem BI can be found in literature and our results only confirm this biological efficiency (Kraus *et al.* 1981; Kraus 1986; Pavela 2007), not so many sources are available on the insecticide efficiency of BI based on *P. pinnata*, and therefore the results presented herein are very precious and new for the science.

Several authors found direct insecticide effects on some pests (Kumar & Daniel 1981; Bhatnagar & Sharma 1995; Hussain *et al.* 1996; Kulat *et al.* 1997). Generally, the pongam oil is critical for antifeedance, repellence or deterrence for pest species. For example, extracts from seeds of *P. pinnata* showed an antifeedant effect on *Diacrisia obliqua* Walker (Chakraborty & Roy 1988; Mohanty *et al.* 1988), *Scelodonata strigicollis* Mots. (Durairaj *et al.* 1991), *Spodoptera litura* F. and *Tribolium castaneum* Herbst (Prakash & Rao 1986) and *Trialeurodes vaporariorum* (Pavela 2008). Repellent and antifeedant effects are often connected with pest reduction or oviposition deterrence effect (Deka *et al.* 1998, Pavela & Herda 2007a,b). Moreover, *P. pinnata* seed oil shows significant fungicide effects (Kumar & Kalidhar 2003).

Unlike pyrethrum, substances contained in *P. pinnata* and *A. indica* extracts are considered to be selective. It is possible to extract these substances if they have good environmental stability and are environmentally safe, and to use these extracts as botanical insecticides in plant protection. For this reason, botanical insecticides can be recommended as suitable to provide the protection of vegetables against common phytophagous pests in all crop growing systems.

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