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Oviposition-deterring activity of sixteen extracts of medicinal plants, extensively used in modern medicine, against *Cydia pomonella* L. (Lepidoptera: Tortricidae)

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In dual-choice tests with sixteen extracts of medicinal plants, six extracts (conc. 2%) reduced the oviposition of codling moth females significantly at the 1% level, and three at the 5% level. Three of five promising extracts gave significant results also when tested under no-choice conditions. The extracts of *Artemisia absinthium* and the green husks of *Juglans regia* gave significant oviposition-deterring effects, even at the low concentration of 0,25%.

Research on oviposition-deterring materials against phytophagous insects dates back to the work of HAMMOND (1929) and LIPP (1929, 1934). However, substantial research in this field appears to have not been carried out before PROKOPY (1972) reported the existence of a marking pheromone in apple maggot flies, *Rhagoletis pomonella* WALSH (Diptera: Tephritidae), that deters repeated oviposition in already infested fruit. Since then quite a number of papers dealt with oviposition deterring pheromones, deposited by the females of phytophagous insects, mainly tephritids and specially *Rhagoletis* species, on the fruits after oviposition (reviews by PROKOPY, 1981 a, b). However, some publications also report on the oviposition-deterring activity of some organic and inorganic substances (CHAPMAN, 1974, SMITH *et al.* 1974, SARINGER 1976, JERMY & SZENTÈSI, 1978; RENWICK & RADKE, 1980; REISSIG *et al.* 1983).

Although it has been found that phytochemicals play an important role in plant-insect relationships, and may serve as pheromone precursors (VITÉ & FRANCKE, 1976) as well as hormones and antihormones in insects (SLAMA, 1979; BRATTSTEN, 1983; SEIGLER, 1983; STIPANOVIC, 1983; MEHROTRA & GUJAR, 1984), only sporadic attempts have been made to study the oviposition-deterring activity of plant extracts or their products against insects (LIPP, 1929; GUPTA & THORSTEINSON, 1960; APPLEBAUM *et al.* 1965; BYRNE *et al.* 1967; HSIAO & FRAENKEL, 1968; SMITH *et al.* 1973; FLINT *et al.* 1977; ALFARO *et al.* 1981; RENWICK & RADKE, 1982; SAXENA & BASIT, 1982; MESSINA & RENWICK 1983; SHARMA *et al.* 1984). Moreover, neither the codling moth (*Cydia pomonella* L.), considered as a "key pest of apple worldwide, with the exception of Japan and parts of Asia" (CROFT, 1982), nor the extracts of medicinal plants (with a few exceptions) known for their safety have been included in any of the foregoing experiments.

Considering the ever-increasing demands for safe alternatives to conventional insecticides, as well as the importance of oviposition as a key in the reproduction of most insects, sixteen extracts of medicinal plants, extensively used in modern

medicine, were evaluated for their oviposition-deterring activity. They were selected on the basis of their safety to man, repellent activity to insects reported previously, availability, and water solubility. This paper reports the results of our studies on the influence of the extracts on the oviposition of *C. pomonella* females.

MATERIALS AND METHODS

Insects

One- to three-day-old adults of the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), reared after the method of HUBER *et al.* (1972) at a constant temperature of 26°C and relative humidity of 60% under continuous illumination, were used throughout the experiments. The insects were first anaesthesized with CO_2, and then three pairs of unmated males and females were picked out for each replicate.

Extracts

The extracts of medicinal plants (Tab. 1) kindly provided by Emil Flachsmann AG, Zürich, were dissolved in distilled water at a rate of 2% (v/v or w/v). The extracts with highly significant oviposition-deterring activity in no-choice experiments were further studied at lower concentrations. Freshly prepared solutions of the extracts were used throughout the studies.

Bioassays

Dual-choice tests

Each diluted extract was painted with a soft brush on the half of the inner surface of a transparent-plastic cap, 200 ml in capacity, at a rate of ½ ml/cap. To avoid contamination, the caps were laid on the treated sides, air-dried in shade and at room temperature (22 ± 1°C). The opposite side of each cap was treated in a similar manner with distilled water alone, and was used as control. The insects were introduced to the bottom of each cap, and the lid was closed immediately. To eliminate fumigant effects of the extracts and to supply the insects with fresh air, the lids were adequately perforated. The caps were transferred into an air-conditioned room with constant temperature of 25°C, 60% relative humidity, and a 16L: 8D light regime. The rate of oviposition was determined after 10 days, when all of the insects were dead. For the evaluation of the oviposition-deterring activity of each extract an anti-ovipositant index I corresponding to the percentage inhibition of oviposition was calculated by the following formula:

$$I = 100 \times \frac{E_c - E_e}{E_c}$$

where E_c is the number of eggs laid on the water-treated surfaces (controls) and E_e the eggs laid on extract-treated surfaces. The experiments were conducted with a completely randomized design, and the treatments were replicated five times.

Tab. 1. General characteristics of sixteen extracts of medicinal plants[1], extensively used in modern medicine, used in the oviposition-deterring studies against *Cydia pomonella* females.

Extracted plants		Parts extracted	Solvent	Form	Yield[2]
Scientific name	Common name				
Angelica archangelica L. (Umbelliferae)	angelica	roots	ethanol	liquid	3:1
Artemisia absinthium L. (Compositae)	wormwood (absinth)	foliage	water	liquid	4:1
Calendula officinalis L. (Compositae)	marigold	flowers	water	liquid	1:1
Eucalyptus sp. (Myrtaceae)	eucalyptus	leaves	water	liquid	4:1
Foeniculum vulgare Mill. (Umbelliferae)	fennel	fruits	water	powder	6:1
Gentiana lutea L. (Gentianaceae)	gentian	roots	ethanol	liquid	3:1
Juglans regia L. (Juglandaceae)	Persian walnut	leaves	Propylene glycol	liquid	1:1
Juglans regia L. (Juglandaceae)	Persian walnut	fruits (outer green layer)	water	liquid	4:1
Lavendula vera DC. (Labiatae)	lavender	flowers	Propylene glycol	liquid	1:1
Marrubium vulgare L. (Labiatae)	horehound (marvel)	foliage	water	liquid	4:1
Melissa officinalis L. (Labiatae)	common balm (balm mint)	foliage	water	liquid	3:1
Mentha piperita L. (Labiatae	peppermint	leaves	water	liquid	3:1
Rheum officinale H.Bn. (Polygonaceae)	rhubarb	rhizomes	ethanol	powder	5:1
Rosa canina L. (Rosaceae)	hip (haw)	fruits	water	powder	2:1
Valeriana officinalis L. (Valerianaceae)	valerian	roots	ethanol	powder	4:1
Viscum album L. (Loranthaceae)	mistletoe	foliage	water	liquid	4:1

[1] Based on Karrer (1976), Zargari (1982), and the information supplied by Emil Flachsmann AG, Zürich

[2] (Dry matter: Extract)

No-choice tests

The extracts with highly significant oviposition-deterring activity, were also studied in no-choice tests. The inner surface of plastic caps, entirely painted with aqueous solutions of the extracts or distilled water alone, was first air dried and then used as an oviposition site for the insects. All other methods were similar to those of the dual-choice tests mentioned in the foregoing section.

Dual-choice tests

Tab. 2 summarizes in the first column the oviposition-deterring activity of sixteen extracts of medicinal plants against *C. pomonella* females at 2% conc. in the dual-choice experiments. The extracts of *J. regia* (green husks), *A. absinthium, V. alba, F. vulgare, C. officinalis,* and *M. piperita,* when painted on the inner surface of the caps, respectively resulted in 71, 70, 59, 56, 48, and 45% inhibition of oviposition on the treated sides. Lower oviposition-deterring activity, at the 5% level, was also observed in *L. vera* (31%), *M. vulgare* (30%), and *G. luteola* (28%). The remaining extracts showed no significant effect on the oviposition.

No-choice tests

Tab. 2 reports in the second column the results of the oviposition-deterring activity of five of the promising extracts, under the no-preference conditions.

Tab. 2. Oviposition-deterring activity of respectively sixteen and five extracts of medicinal plants against *Cydia pomonella* females, at a 2% conc., in dual-choice and no-choice-experiments.

Source of extracts	Antiovipositant index[a] (% inhibition)	
	dual-choice exp.	no-choice exp.
Angelica archangelica	16	-[b]
Artemisia absinthium	70**[c]	44**
Calendula officinalis	48**	33**
Eucalyptus sp.	5	-
Foeniculum vulgare	56**	-
Gentiana luteola	28*	-
Juglans regia (leaves)	15	-
Juglans regia (green husks)	71**	51**
Lavendula vera	31*	-
Marrubium vulgare	30*	-
Melissa officinalis	8	-
Mentha piperita	45**	16*
Rheum officinalis[d]	16	-
Rosa canina[d]	1	-
Valeriana officinalis[d]	17	-
Viscum album	59**	2
Control (distilled water)	0	0

[a] Average of five replicates, assayed during the life span of the adults. For formula of index see text.

[b] Not tested

[c] Student's t test on difference between treated and control indicated by: *p <0.05, **p <0.01

[d] Due to low solubility in distilled water, 0.5% conc. was used

Ovipositions of *C. pomonella* females on the inner surface of the caps, entirely painted with the extracts of *J. regla* (green husks), *A. absinthium,* and *C. officinalis,* was highly inhibited at the rates of 51, 44 and 33%, when compared with the corresponding controls. *M. piperita* also resulted in a significant reduction (16%), while *V. alba* did not exhibit a pronounced activity.

Lower concentrations

The oviposition-deterring activity of the lower concentrations of the most promising extracts, under dual-choice conditions, are summarized in Tab. 3. While the anti-ovipositant index of *A. absinthium* extract remained over 50%, even at 0.25% concentration, the activity of *J. regia* husk-extract was drastically reduced, from 79 to 17%, at the lower concentrations. Moreover, the extract of *C. officinalis* lost its oviposition-deterring activity at the concentration of 0.5% and significantly stimulated the oviposition at the lowest concentration.

Tab. 3. Oviposition-deterring activity of three extracts of medicinal plants against *Cydia pomonella* females, at four concentrations, in dual-choice experiments (otherways as in Tab. 2).

% conc. (v/v)	Antiovipositant index (% inhibition)		
	A. absinthium	*C. officinalis*	*J. regia*[a]
2	67**	37**	79**
1	59**	21*	50**
0.5	52**	-3	35**
0.25	56**	-20*	17*
Control	0	0	0

[a] (green husks)

DISCUSSION

None of the previous papers appear to have reported the oviposition-deterring activity of our experimental extracts, listed in Tab. 1, against *Cydia* species. The only research works with these extracts, or the corresponding plants, in this field, seem to be those of KOEHLER *et al.* (1983), GUPTA & THORSTEINSON (1960), and SAXENA & BASIT (1982). LIPP (1929) appears to be the only person to report the oviposition-deterring activity of a substance against a species of *Cydia,* whereas SCHMID & HENGGELER (1979) and KREUTER (1983) report the use of *A. absinthium* in popular gardening.

KOEHLER *et. al.* (1983) while evaluating the significance of the use of several plants, including marigold (*Calendula officinalis*) as companion plants in popular gardening, found out that the oviposition of the cabbageworm (*Pieris rapae* L.) on cabbage was significantly reduced when marigold, nasturtium (*Tropaeolum* sp.) or catnip (*Nepeta cataria*) were planted at a rate of four plants/experimental plot. However, they also observed that the beneficial effects of the companion plants

were negated by substantial reductions in cabbage yield, either as a result of competition for resources or allelopathic action. GUPTA & THORSTEINSON (1960) have reported the oviposition-deterring activity of marigold plants against the diamondback moth, *Plutella maculipennis* (CURT.).

The oviposition-deterring activity of *C. officinalis* extract against codling moth (a Tortricid), that of the plant against the diamondback moth (a Plutellid), and as a companion plant against the cabbageworm (a Pierid), suggest, potentially, the presence of the deterrent activity of the extract towards a wide range of insects. That the lowest concentration (0.25%) of this extract stimulates oviposition, should not be overlooked. A *C. officinalis* extract that initially deters the oviposition, may later (after a substantial decomposition of the residues) act as an oviposition stimulant.

Although SAXENA & BASIT (1982) have reported the inhibition of oviposition by volatiles of certain plants, including eucalyptus, in a leaf hopper – *Amrasca devastans* (DISTANT) – the aqueous solutions of eucalyptus extract did not result in a significant reduction of egg-laying in codling moth, when tested under our experimental conditions. The difference in the test insect or other experimental conditions may account for the lack of activity.

According to LIPP (1929) the odor of α-naphthylamine sprayed onto peach twigs prevented oviposition by the oriental peach moth *Cydia molesta* for up to seven days. Alkaloids reduced oviposition of this insect on the larval host-plant. The possibility of similar activity of these chemicals on *C. pomonella* remains to be examined.

Although nine of the extracts significantly deterred the oviposition of the codling moth in dual-choice tests (Tab. 2), the extracts of *A. absinthium, C. officinalis,* and *J. regia* (husks) deserve special attention. These extracts exhibited a highly significant oviposition-deterring activity in no-choice tests, i.e. in the absence of a non-treated oviposition-site (Tab. 2), *A. absinthium* and *J. regia* also at much lower concentrations (Tab. 3).

A. absinthium is recommended in popular gardening as an oviposition-deterring material to control *Pieris brassicae* L. and *C. pomonella* (KREUTER, 1983). According to SCHMID & HENGGELER (1979), spraying *A. absinthium* tea in June and July on apple trees, during the flying time of the codling moth, checks the infestation. Interestingly several applications of the same preparation on cherry trees, three weeks after formation of the flowers, are recommended to biological gardeners in order to deter the oviposition of *Rhagoletis cerasi* L., too (KREUTER, 1983; SCHMID & HENGGELER, 1979). The promising results of KATSOYANNOS & BOLLER (1976, 1980) in the field application of the oviposition-deterring pheromone of the same insect, sprayed several times, during a similar period of the year, suggest the possible involvement of a pheromonal mimic in the oviposition-deterring activity of *A. absinthium* extract.

The presence of oviposition-deterring activity in the extract of the green husk of Persian walnut, *J. regia,* against the codling moth, which also attacks Persian walnut (BOYCE, 1935; METCALF & FLINT, 1962; MICHELBACHER, 1945), is surprising. However, according to BOYCE (1935), the codling moth attacking walnut in California may be a physiologically or otherwise different ecotype.

Considering the complexity of the chemical components present in the extracts (KARRER, 1976; ZARGARI, 1980), no conclusive remarks can be made before isolation of the active ingredients. However, according to our laboratory investigations, the results are promising enough to justify further research on the extracts,

both under laboratory and field conditions, with improved methods and materials. Moreover, in designing the experiments, the practical aspects of the problems and prospects of oviposition-deterring substances, discussed by KATSOYANNOS & BOLLER (1976, 1980), PROKOPY (1972, 1981 a, b) and FLETCHER (1977), should be taken into consideration.

ZUSAMMENFASSUNG

Zweiprozentige Lösungen von 16 Extrakten von Medizinalpflanzen wurden an Weibchen des Apfelwicklers (*Cydia pomonella*) auf ihre ovipositionshemmende Wirkung geprüft. In Wahlversuchen (unbehandelt/behandelt) ergaben 9 dieser Extrakte eine signifikante Reduktion der Eiablagerate, und zwar bei 3 mit $P < 0.05$ und bei 6 mit $P < 0.01$ (Tab. 2). Von den letzteren wurden 5 Extrakte auch im Nichtwahltest geprüft, wobei die Extrakte von *Artemisia absinthium,* grünen Fruchtschalen von *Juglans regia* und *Calendula officinalis* wiederum eine gut signifikante Hemmung der Eiablage ergaben ($P < 0.01$). Die beiden erstgenannten Extrakte ergaben auch in 0.5- und 0.25prozentiger Konzentration noch signifikante Hemmung (Tab. 3), während der Extrakt von *C. officinalis* in niedriger Konzentration die Eiablage förderte.

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