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Tests with the extracts of 21 medicinal plants for antifeedant activity against larvae of Pieris brassicae L. (Lep., Pieridae)

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The extracts of 21 popular medicinal plants have been screened for antifeedant activity against larvae of *Pieris brassicae* L. The dual-choice and no-choice studies revealed significant antifeedant activity of the *Mentha piperita* L., *Angelica archangelica* L,, *Eucalyptus sp,*, *Arthemisia absinthium* L., and *Melissa officinalis* L. extracts.

The idea of using antifeedants, feeding deterrents, in plant protection is not new and at least dates back to BRINELY (1926). Antifeedants may be used safely to spray plants against insects, without harming parasites, predators or pollinators (MUNAKATA, 1970). They may be also further studied for breeding resistant varieties of plants by selecting for inhibitory attributes (CHAPMAN, 1974), which appears to be an ideal solution to many pest control problems (RENWICK, 1983).

During the last decade at least two comprehensive reviews (CHAPMAN, 1974; VIGNERON, 1978) and three book chapters (KUBO & NAKANISHI, 1977; MUNAKATA, 1977; NORRIS, 1977), exclusively on the chemical inhibition of feeding by phytophagous insects, have been published. From over 300 articles reviewed by CHAPMAN (1974) and VIGNERON (1978), about 7% of them have appeared before 1950, 8% during 1950-1959, 40% in 1960-1969, and 45% within 1970-1978. Sudden increase in antifeedant research in the sixties coincides with the drastic change in the public attitudes towards conventional pesticides in this period (METCALF, 1980) and the public demands for safe substitutes, which in turn resulted in concerted efforts to develop safe alternatives, including antifeedants. Moreover, numerous entomologists have been motivated to contribute to better understanding of antifeedants as a promising alternative in insect control (BERNAYS & CHAPMAN, 1977, 1978; BRATTSTEN, 1983; BURNETT & JONES, 1978; HEDIN *et al.*, 1977; KOGAN, 1976; MA, 1972; MARBY *et al.*, 1977; RENWICK, 1983; SEIGLER, 1983; STIPANOVIC, 1983).

Recently, special efforts have been made to screen materials of plant origin for their antifeedant activity (MUNAKATA, 1970, 1977; REED *et al.*, 1981), for - as the latter state - "such screening is important in discovering safe, biodegradable alternatives to synthetic insecticides". Surprisingly, the medicinal plants, which have stood the test of time and the modern medicine for their safety, have received little, if any, attention. The Indian neem tree (*Azadirachta indica* A. JUSS of the Meliaceae family), an old medicinal plant of India which is rarely used against fever (URBAN, 1977), appears to be the only medicinal plant extensively used in the screening programs for its antifeedant activity (JACOBSON, 1958, 1975, 1981; KRAUS *et al.*, 1981; REED *et al.*, 1982; WARTHEN, 1979). Therefore, additional efforts for inclusion of the most popular medicinal plants in the screening pro-

Table 1: General characteristics of twenty-one extracts of medicinal plants[1], extensively used in modern medicine, used in the antifeedant studies against P. brassicae larvae.

Extracted plants		Parts extracted	Solvent	Form	Yield (Dry matter:Extract)
Scientific name	Common name				
Allium cepa L. (Liliaceae)	onion	bulbs	water	liquid	3:1
Allium sativum (Liliaceae)	garlic	bulbs	water	powder	3:1
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
Equisetum arvense L. (Equisetaceae)	horse-tail	foliage	ethanol	liquid	4:1
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[2]	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
Matricaria chamomilla L. (Compositae)	German chamomile	flowers	water	powder	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
Urtica dioica L. (Urticaceae)	stinging nettle	leaves	water	powder	5:1
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] Outer green layer.

grams, are justifiable. With the present paper we report on the antifeedant activity of 21 extracts of medicinal plants (extensively used in modern medicine) against *Pieris brassicae* larvae. The extracts were selected on the basis of the following criteria: 1) Safety, based on the recent pharmacopoeia of Switzerland (NEUGEBAUER & MORANT, 1983), 2) availability, based on the lists of relevant companies (ANONYMOUS 1983) and the possibility of cultivation of the original plant (EBERT, 1949; FUEK, 1980; MUELLER, 1982; SCHMID & IMPHOF, 1982; WEIDINGER, 1983), 3) water solubility, and 4) previous reports, if any, on their application as insecticides or insect repellents (BERGHAMMER, 1982; FUHRMANN, 1935; KREMER, 1981; KREUTER, 1983; MARZELL, 1958; SCHMID & HENGGELER, 1979; TREBEN, 1980).

MATERIALS AND METHODS

Dual-Choice Studies.

Third instar larvae of *Pieris brassicae* L. were collected soon after molting from the department colony, reared on cabbage, and starved 24 h at room temperature (22 ± 1 °C) before testing.

The extracts of medicinal plants (Table 1), generously provided by Emil Flachsmann AG, Zurich, were dissolved in distilled water at a rate of 2% (v/v or w/v). Lower concentrations (1%, 0.5% and 0.25%) were also assayed, when a promising activity was observed. Freshly prepared solutions were used throughout the experiments.

Leaf discs, 20 mm in diameter, were punched out with a cork borer from the young leaves of *Brassica oleracea var. capitata alba.* The discs were dipped into the diluted solutions, or into distilled water, as a control, for 2 seconds, shaken to remove excess liquid and subsequently air dried in the shade and at room temperature.

A paper towel, 135 x 70 mm, was placed on the bottom of a polyethylene box, 135 x 70 x 35 mm. Then, two treated and two control discs were put on the paper, in the middle of the box, so that each disc was located about 10 mm from its adjacent disc.

Five larvae were introduced to the center of the box, and the lid was closed immediately. To eliminate fumigant effects of the extracts, if any, each box was aerated by 6 circular openings, 10 mm in diameter, covered with a looseweave muslin, in a symmetrical arrangement. The dishes were transferred into an air-conditioned room with constant temperature of 25 °C and 60% relative humidity. The consumed areas of all discs were measured by DETHIER's method (DETHIER, 1947), after 24 h. The percentage of feeding inhibition was determined by the following formula:

$$\text{Antifeedant index (AFI)} = \frac{C - T}{C} \times 100$$

where C is the consumed area of the control disc and T the consumed area of treated disc. The experiments were conducted with a completely randomized design, and the treatments were replicated five times.

No-Choice Studies.

For the extracts with highly significant feeding-inhibitory activity, the larvae were given a no-choice test in separate polyethylene boxes containing two treated or two control discs, placed in the middle of the boxes with a distance of about

2 cm to evaluate their efficacy in the absence of preferable foods. All other methods were similar to those in the dual-choice studies, mentioned earlier.

RESULTS

Dual-Choice Studies.

Table 2 summarizes the antifeedant effect of 21 extracts of medicinal plants against *P. brassicae* larvae, at a concentration of 2%, in the dual-choice experiments. Twenty-hour feeding of the third stage larvae of this insect on the treated and control discs revealed the presence of significant feeding-stimulant or feeding-deterrent activities almost in all of the extracts used in our experiments. While the extracts of the bulbs of *A. cepa* and the leaves of *J. regia* stimulated the feeding significantly, the extracts of *A. archangelica, Eucalyptus sp.,* and *M. piperita* resulted in 100% inhibition.

Table 2: Antifeedant activity of 21 extracts of medicinal plants against Pieris brassicae larvae, at a 2% concentration, in the dual-choice experiments.

Source of extracts[a]	AFI[b]	Source of extracts	AFI
Allium cepa	-32 *[c]	*Lavendula vera*	17
A. sativum	-16	*Marrubium vulgare*	80 **
Angelica archangelica	100 **	*Matricaria chamomilla*[d]	55 **
Artemisia absinthium	90 **	*Melissa officinalis*	96 **
Calendula officinalis	50 **	*Mentha piperita*	100 **
Equisetium arvense	29 *	*Rheum officinale*[d]	35 *
Eucalyptus sp.	100 **	*Rosa canina*[d]	10
Foeniculum vulgare	62 **	*Urtica dioica*	33 *
Gentiana lutea	81 **	*Valeriana officinalis*[d]	34 *
Juglans regia (leaves)	-46 **	*Viscum album*	31 *
J. regia (outer layer of fruits)	26 *	Control[e]	0

[a] For further information please refer to Table 1.

[b] Antifeedant index (% inhibition); average of five replicates, assayed after 24 h of feeding (see text).

[c] * and ** = significantly different from its corresponding control at respectively 5% and 1% level, based on paired t-test.

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

[e] Formulation blank consisting of distilled water only.

Extracts of *M. officinalis, A. absinthium, G. lutea, M. vulgare, F. vulgare, M. chamomilla,* and *C. officinalis* respectively reduced the feeding at the rates of 96%, 90% 81%, 80%, 62%, 55%, and 50%, in comparison to the corresponding control at the 1% level. The remaining extracts, with the exception of *A. sativum, L. vera,* and *R. canina* which were not significantly active, also exhibited some antifeedant activity at the 5% level.

Table 3: Feeding inhibitory activity of nine promising extracts of the medicinal plants against Pieris brassicae larvae, at a 2% concentration, in no-choice experiments (otherwise as in Tab. 2).

Source of extracts	Antifeedant-index (% inhibition)
Angelica archangelica	69 **
Artemisia absinthium	19 *
Calendula officinalis	4
Eucalyptus sp.	58 **
Gentiana lutea	12
Marrubium vulgare	9
Matricaria chamomilla[d]	5
Melissa officinalis	20 *
Mentha piperita	73 **
Control	0

No-Choice Studies.

Table 3 reports the inhibitory activity of 9 promising extracts of the medicinal plants against *P. brassicae* larvae, at a concentration of 2%, in no-choice experiments. Extracts of *M. piperita, A. archangelica,* and *Eucalyptus* sp. significantly reduced the feeding at the rates of 73%, 69%, and 58%, at the 1% level. However, the extracts of *M. officinalis,* and *A. absinthium,* under similar conditions, resulted in a lower, but significant rate of inhibition. The remaining extracts exhibited no significant effect on feeding.

Lower concentrations.

Table 4 summarizes the results of lower concentrations of 5 promising extracts of the medicinal plants under dual-choice conditions. The lowest concentration of 0.25% of *M. piperita* and *A. archangelica* resulted in the highly significant

Table 4: Antifeedant activity of five extracts of medicinal plants against Pieris brassicae larvae, at four concentrations, in the dual-choice experiments (otherwise as in Tab. 2).

Source of extracts	Conc. %	AFI
Angelica archangelica	2	100 **
	1	100 **
	0.5	88 **
	0.25	71 **
Artemisia absinthium	2	100 **
	1	78 **
	0.5	71 **
	0.25	10
Eucalyptus sp.	2	100 **
	1	100 **
	0.5	90 **
	0.25	48 **
Mentha piperita	2	100 **
	1	99 **
	0.5	88 **
	0.25	75 **
Melissa officinalis	2	96 **
	1	89 **
	0.5	70 **
	0.25	35 *
Control		0

feeding reductions of 75% and 71% respectively, whereas for the extracts of *A. absinthium* and *M. officinalis* a twice as high concentration was required to result in ca. 70% reduction. The extract of *Eucalyptus* sp. showed intermediate antifeedant activity.

DISCUSSION

None of the previous research appears to have reported the antifeedant activity of our experimental extracts (Table 1) against *Pieris* spp. However, absinthin, present in *A. absinthium,* (MUNAKATA, 1977; VIGNERON, 1978; WADA &

MUNAKATA, 1971), juglone, present in the fleshy green part of the *J. regia* fruit (MUNAKATA, 1977, REED *et al.*, 1981), and the water extracts of *Eucalyptus* sp., and *U. dioica* (BERNAYS & CHAPMAN, 1977) have been reported to have antifeedant activity against *Spodoptera* spp., *Scolytus* spp. and *Locusta migratoria* (L.), respectively. Moreover, according to SCHMID & HENGGELER (1979) application of the extracts of *E. arvense* against aphids and mites, and of *A. absinthium* against ants, caterpillars, aphids, codling moth, and mites, is recommended in biological gardening. However, the mechanism(s) of their action or the possibility of their antifeedant activity have not been reported by these authors.

Antifeedant activity of different compounds (sodium chloride and nitrate, calcium salts, berberin hydrochloride, conessine, ecdysterone, inokosterone, morphine hydrochloride, ponasterone A, quinine chloride, hydrochloride or sulphate, solanine, sparteine, strychnine nitrate, and tomatine) against *P. brassicae* larvae has been reported (CHAPMAN, 1974; MA, 1972). Of the twenty-two compounds, mainly alkaloids and related compounds, tested by MA (1972), the most effective antifeedant materials against. *P. brassicae* larvae "possessed an alkaloidal or steroidal structure of high molecular weight." Antifeedant activity of azadirachtin, the active material of the neem tree *Azadirachta indica,* against *P. brassicae* larvae has been studied by BUTTERWORTH & MORGAN (1971). While this compound completely inhibited feeding in the desert locust, *Schistocerca gregaria,* it showed only moderate activity against the larvae of *P. brassicae.* Since most of the compounds reported by MA (1972) and CHAPMAN (1974), with the exception of the inorganic salts, are either insoluble or slightly soluble in water (WEAST, 1976), their possible involvement in the antifeedant activity of water extracts used in our studies (Table 1) is questionable. However, due to the complexity of compounds present in the original plants (KARRER 1976), no conclusive remarks could be made before isolation and identification of the active substances.

Under the test conditions used, none of the extracts caused a mortality during or after a 24-h period of contact. Moreover, some of the larvae started feeding on the treated discs after finishing the control discs, when they remained more than 24 h in the polyethylene dishes. Therefore, although it is believed that in the field the insects leave the treated plants, wander elsewhere, find weed plants to eat or die of predation or starvation (WRIGHT, 1967), the possibility of habituation, explained by CHAPMAN (1974), should not be overlooked in field studies.

That *P. brassicae* larvae, which normally select their food by the presence of characteristic stimulatory chemicals rather than by the absence of inhibitors (MA, 1972), were highly influenced by many of the extracts tested in our experiments, is promising. Therefore, potentially, these extracts may also exhibit antifeedant activity against many other phytophagous insects.

Considering the high efficacy of extraction, expressed as the ratio of dry matter to the extract (Table 1), highly significant activity of nine extracts in dual-choice tests, (Table 2), their activity in no-choice studies (Table 3), and the low concentrations needed for obtaining relatively high antifeedant activity (Table 4), further studies for determination of the practical significance of these findings are justifiable.

ZUSAMMENFASSUNG

Extrakte von 21 Medizinalpflanzen wurden auf ihre frasshemmende Wirkung auf Raupen des Grossen Kohlweisslings (*Pieris brassicae* L.) geprüft. Bei Doppel-Wahlversuchen wurden je 2 behandelte und 2

unbehandelte Kohl-Blattrondellen von 2 cm Durchmesser in Polystyrenschachteln (135 x 70 x 35 mm) für 24 h je 5 Raupen zum Frass angeboten. Daneben wurden auch einfache Frasstests (ohne Wahlmöglichkeit) durchgeführt, indem den Raupen je Schachtel nur entweder 2 behandelte oder 2 unbehandelte Kohlrondellen geboten wurden. Die Wasserextrakte von Pfefferminz (*Mentha piperita* L.), *Eucalyptus sp.*, , Wermut (*Arthemisia absinthia* L.) und Melisse (*Melissa officinalis* L.) sowie der Alkoholextrakt von Enggel-Brustwurz (*Angelica archangelica* L.) ergaben in Konzentrationen von 0,25–0,5% signifikante Frassshemmung. Die Versuche rechtfertigen es, weitere Untersuchungen über die Wirkung solcher Extrakte im Feld durchzuführen, um damit mehr über eine allfällige praktische Bedeutung zu erfahren.

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