Toxicity of Individual Pyrethrin Esters to House Flies (Diptera: Muscidae)[1]

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Abstract Six pyrethrin esters were separated from whole pyrethrum by using high performance liquid chromatography on a silica column. Dilutions of individual esters were applied topically to house flies (*Musca domestica* L.) and compared to whole pyrethrum and trans-permethrin. LD_{50}'s averaged from two significant dosage-mortality regressions per chemical were in ascending toxicity: Cinerin I (1.77 µg/fly), jasmolin I (1.28 µg/fly), pyrethrin II (0.49 µg/fly), jasmolin II (0.46 µg/fly), cinerin II (0.43 µg/fly), pyrethrin I (0.20 µg/fly), 25% pyrethrin extract (0.11 µg/fly) and trans-permethrin (0.0072 µg/fly).

Key Words Pyrethrin, house fly, pyrethrum, *Musca domestica*

Pyrethrum extract used in various products is a complex mixture of compounds including the six pyrethrin esters that are responsible for the insecticidal activity. The six esters are structurally similar to each other and consist of an acid moiety and an alcohol moiety. The acid moiety is either chrysanthemic acid (I's) or pyrethric acid (II's); the alcohol or rethrolene moiety is either pyrethrolone (pyrethrin I and II), cinerolone (cinerin I and II), or jasmolone (jasmolin I and II). A detailed chemistry of pyrethrins is given by Crombie (1995).

Pyrethrins have largely been displaced in commercial applications by synthetically produced pyrethroids. These compounds are typically photostable, more efficacious than the natural pyrethrins, and cheaper to produce. Yet, the natural pyrethrins retain a significant niche in the market for human and animal health care and for in-home insect sprays. Their relatively short-lived toxicity is advantageous for these applications. The pyrethrin market has remained fairly stable in the past 2 decades and could be expanded if costs of production were lowered and if a reliable supply was available. The tools of biochemistry and molecular biology have recently been employed in an effort to find a means of improving and stabilizing the supply of pyrethrins. The gene for chrysanthemyl diphosphate synthase has been cloned and expressed in *Escherichia coli* (patent #5443978). This, along with more economical means of chemical production of the rethrolones, is leading towards viable commercial production of individual esters of pyrethrins.

Previous efficacy reports of the individual pyrethrin esters on house flies (*Musca domestica* L.) do not contain data for all six esters. Ranking of the esters for efficacy must be made by extrapolation. By combining data from several reports the ranking

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in ascending toxicity to house flies is jasmolin II, jasmolin I, cinerin II, cinerin I, pyrethrin II, and pyrethrin I (Gersdorff 1947, Incho and Greenberg 1952, Broadbent and Hagarty 1969, Chang and Kearns 1962, Matsuia and Meguro 1964, Godin et al. 1965, Moore 1975, Ando et al. 1983). Some studies have ranked pyrethrin II over pyrethrin I (Sawicki et al. 1962, Sawicki and Elliot 1965).

Because the commercial production of all of the esters is probably not economically feasible, it is important to determine which esters to produce. There are no studies available that have compared all six esters. Therefore, these studies were conducted to determine which of the esters would be most appropriate for commercial production.

Materials and Methods

Refined pyrethrum extract (20 to 25% total pyrethrin, Fluka Chemical Co., St. Louis, MO) was separated by several silica columns for chromatographic separation. An initial separation of the I's from the II's and removal of some of the aliphatic material found in pyrethrum extract was accomplished by eluting whole extract through Kiesel Gel (Merck and Co., Inc., Whitehouse Station, NJ) using hexane:iso-propanol (90:10) in a gravity-fed solvent system. Fractions from this separation were run on a preparative scale μPorasil® column (Waters Corp., Milford, MA) on HPLC also using a hexane:iso-propanol solvent system (99.5:0.5 or gradient). Solvent was removed from the purified esters by distillation under reduced pressure. The esters were resuspended in d-chloroform and subjected to nuclear magnetic resonance (NMR) analysis to assess purity.

Separated esters were shipped from Salt Lake City to Tifton while being chilled in insulated containers. Once in Tifton they were refrigerated until used. Rigorous dose determinations first were conducted with 25% pyrethrum extract. This extract was diluted with acetone to produce six doses of pyrethrum ranging from 0.800 to 0.025 μg per fly. Each dose was tested against 60 house flies. Preliminary range finding had indicated that these doses should produce responses from 0 to 100% mortality. The pyrethrum mean LD_{50} resulting from two tests was used as the midpoint in a series of seven doses used in a preliminary test for each ester, with 10 flies per dose. After these tests, seven dose levels expected to produce 0 to 100% mortality were applied to 60 flies per dose. Dose-level samples were prepared in amber glass vials and used the same day.

The house fly strain used was the Cornell-susceptible strain (CS) (Tomita et al. 1995). A colony was established in Tifton, and progeny were used in these tests. Adults were fed water and a dry mixture of sugar and powdered milk (1:1). Larvae were reared on CSMA fly larvae media (PI Feeds Inc., St. Louis, MO). Mixed sex flies were tested when 3 to 4 d old.

All toxicants were tested using the following procedures. Flies were removed from holding cages with a battery powdered aspirator (Hausherr's Machine Works, Toms River, NJ), anesthetized with CO_2, and transferred to a 470-ml waxed paper cup (Sweetheart Cup Corp., Chicago, IL). Toxicant was applied to the fly's prothorax in a 1-μl droplet of acetone solution from a Hamilton PB600 microliter syringe (Hamilton Co., Reno, NV). Dosed flies were held in the same 470-ml cup along with a dental wick containing 10% sucrose-water solution. A clear plastic lid was used to close the cup before the flies fully recovered from anesthesia. Three cups of 20 flies (60) were treated for each of 7 dose-levels and a check within each test. Cups were held in a dark room (to prevent photodegradation of the esters) at 26°C, and mortality was

recorded after 24 h. A fly which could not stand was considered dead. Dosage-mortality regressions were calculated by probit analysis (Russell et al. 1977). The test was conducted a second time for each pyrethrin ester as well as pyrethrum and trans-permethrin, and the mean LD_{50} was determined for each treatment.

Results and Discussion

Pyrethrin I showed the greatest efficacy, followed by cinerin II, jasmolin II, and pyrethrin II, all of which were about ⅓ the efficacy of pyrethrin I (Table 1). Jasmolin I and cinerin I were about ⅙th and ⅑th as effective as pyrethrin I, respectively.

Our results differed from some previous reports. Jasmolin II showed better activity than in many earlier reports, and cinerin I performed more poorly. A direct comparison of LD_{50}'s reported by other workers using similar test methodology (Ando et al. 1983, Chang and Kearns 1962), shows good agreement with our results. Ando et al. (1983) reported on pyrethrin I, cinerin I, and jasmolin I. Chang and Kearns (1962) reported on pyrethrin I and II, and cinerin I and II. Both reports place pyrethrin I as the most toxic to house flies, as did we, although our LC_{50} of 0.20 μg/fly is about half of the values they reported. Ando et al. (1983) reported a pyrethrin II LC_{50} of 0.72 μg/fly

Table 1. LD_{50}'s (μg/fly) and slopes of dosage mortality regressions of trans permethrin, pyrethrin esters and 25% pyrethrum extract on house fly

Treatment	Test 1		Test 2		Average
	LD_{50}	Slope	LD_{50}	Slope	LD_{50}
Trans permethrin	0.0071	4.07	0.0073	4.87	0.0072
	(0.0063–0.0081)		(0.0065–0.0087)		
Pyrethrin I	0.18	3.25	0.21	3.24	0.20
	(0.15–0.21)		(0.14–0.31)		
Pyrethrin II	0.36	2.50	0.62	2.82	0.49
	(0.31–0.42)		(0.33–1.01)		
Cinerin I	1.64	3.40	1.90	4.17	1.77
	(1.44–1.89)		(1.67–2.16)		
Cinerin II	0.39	2.81	0.47	4.21	0.43
	(0.33–0.45)		(0.42–0.53)		
Jasmolin I	1.30	3.70	1.25	4.83	1.28
	(1.14–1.47)		(1.10–1.40)		
Jasmolin II	0.38	2.44	0.53	4.92	0.46
	(0.24–0.55)		(0.48–0.59)		
25% pyrethrum	0.10	2.96	0.120	3.08	0.110
extract	(0.08–0.125)		(0.099–0.147)		

* 95% confidence interval.

which is in good agreement with our value of 0.49 µg/fly. These two previous authorities reported cinerin I LC_{50}'s of 1.70 and 1.78 µg/fly, corresponding well with our value of 1.77 µg/fly. Chang and Kearns (1962) reported a cinerin II LC_{50} of 1.73 µg/fly which is greater than our value of 0.43 µg/fly. Ando et al. (1983) gave a jasmolin I LC_{50} of 1.90 µg/fly, which agrees well with our value of 1.28 µg/fly.

Based on our data, pyrethrin I seems to be the most likely candidate for biosynthesis, based on toxicity to the house fly. The next three closely ranked candidate esters for biosynthesis are cinerin II, jasmolin II and pyrethrin II. Jasmolin I and cinerin I are a distant fifth and sixth.

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