Photostabilization of Bioethanomethrin, Resmethrin, and Natural Pyrethrins (Pyrethrum) by Mixed Diaryl-*p*-phenylenediamines

Natural pyrethrins and nonhalogenated synthetic pyrethroids are highly active and safe insecticides but are not photostable. A stabilized formulation was developed based on 1% of mixed diaryl-*p*-phenylenediamines in an aromatic solvent. The synthetic pyrethroids bioethanomethrin and resmethrin as well as natural pyrethrins were protected from degradation when exposed to artificial and solar radiation. The photostabilization effect was also confirmed by bioassay against the western spruce budworm.

The botanical insecticide pyrethrum combines a high insecticidal activity with low toxicity to warmblooded animals. In spite of these favorable qualities, its main use has been limited to the indoors because the active components ("pyrethrins") quickly degrade in sunlight. In several attempts to formulate a more stable product, best results were probably obtained by Miskus et al. (1971). They added antioxidants and ultraviolet (UV) screens. The most promising formulation in the series was produced on a limited scale by the McLaughlin Gormley King Co. as F7076.

Nonhalogenated "pyrethroids" (analogues of pyrethrins), such as bioethanomethrin (5-benzyl-3-furyl *d-trans*-ethanochrysanthemate) and resmethrin [(5-benzyl-3-furyl)-methyl 2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylate, 70% (±)-trans and 30% (±)-cis], also are active insecticides, safe, but photo unstable. More recently some halogenated pyrethroids, such as permethrin [3-phenoxybenzyl (±)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] have shown excellent intrinsic photostability and similar toxicological properties (Elliott et al., 1978).

This paper describes chemical and biological experiments that demonstrate the ability of diaryl-*p*-phenylenediamines to protect two nonhalogenated pyrethroids as well as natural pyrethrins against photodegradation.

MATERIALS AND METHODS

Abbreviations used were as follows: bioethanomethrin, BEM; resmethrin, RES; pyrethrum, PYR; Wing-Stay 100, WS (diaryl-*p*-phenylenediamines); Panasol AN2, PAN (aromatic solvent). All percentages are weight/volume unless stated otherwise.

Thin-Layer Chromatography (TLC). A rapid TLC technique was used to screen 12 potential solvents for a stabilized formulation of BEM. BEM (0.12%) in a test solvent was spotted on a TLC plate (250-μm of silica gel G; Merck) and exposed to a sunlamp (General Electric; Code RS; 275 W) at a distance of 30 cm for 90 min. Development was in hexane–ether (97:3), and spots were visualized by spraying with 3% vanillin in concentrated H_2SO_4 (LeRosen et al., 1952). Percent BEM remaining was estimated by comparing exposed and unexposed spots.

The best solvent, PAN, was used with 0.12% BEM to screen 15 antioxidants and 6 UV absorbers, each at a concentration of 1%.

Exposure on Glass Surfaces and Analysis by HPLC and GLC. Solutions of 0.5% BEM and RES in PAN with 0, 0.2, 0.5, and 1.0% WS were irradiated for 0, 1, 2, 3, and 5 h (sunlamp). Test solutions (0.1 mL) were exposed on a 23 × 54 mm area of a glass slide, placed on a turntable at 30 cm from the light source. Slides were then rinsed with 120 mL of hexane and 40 mL of benzene–hexane (1:1). For cleanup the rinses were passed through a Florisil column (5 cm × 10.5 mm i.d.). After rotary evaporation of the eluate, the residue was dissolved in 75% CH_3CN for HPLC analysis. Operating conditions were as follows: liquid chromatograph, Waters Associates; column, 60 cm × 3 mm o.d., packed with Bondapak C_{18} Corasil (37–50 μm); detector, Tracor, UV variable wavelength, at 229 nm; mobile phase, 75% CH_3CN at 1 mL/min. Quantification was based on peak height. Corresponding unexposed samples served as controls. No attempt was made to separate isomers.

Pyrethrum (PYR) was formulated from Pyrocide 175, an extract containing 20% (w/w) PYR. Solutions (25 μL) of 0.4% PYR in PAN with 0, 0.05, 0.1, and 0.2% WS were exposed on a 24 × 18 mm area of frosted glass. After exposure, the residues were dissolved in benzene for GLC analysis. Operating conditions were as follows: gas chromatograph, Varian Series 2700; column 90 cm × 6 mm o.d. packed with 5% SE-30 on Aeropak 80/100; detector, ^3H electron capture (Donegan et al., 1962; Head, 1966); carrier gas, N_2 at 750 mL/min; temperatures (°C), inlet, 200, column, 200, and detector, 205.

Another formulation of PYR was 1.2% PYR and 1% WS in PAN. Here the standard was F7076, a patented formulation containing 7.2% w/w Pyrocide 175 (1.2% PYR), 0.6% w/w (0.5%) DOPC, 0.6% w/w (0.5%) benzyl cinnamate, 2.4% w/w ethylene glycol monobutyl ether, and 89.2% w/w mineral oil. Control formulations lacked the antioxidants and UV absorber. The above formulations were exposed in 5 cm i.d. Petri dishes (1 mL/dish) to 10 and 17 h of sunlamp radiation at a distance of 30 cm from the light source and to 10 h of sunlight. After exposure the samples were dissolved in benzene for analysis by GLC.

Bioassays. For assessment of the biological effectiveness of the photostabilizers, the four formulations, each with and without stabilizers and each with and without sunlamp exposure, were tested on the western spruce budworm (*Choristoneura occidentalis* Freeman): (1) 0.5% BEM with 1% WS in PAN, (2) 0.5% RES with 1% WS in PAN, (3) 1.2% PYR with 1% WS in PAN, and (4) F 7076. Formulations 1 and 2 were exposed on microscope slides for 4 h and 3 and 4 in petri dishes for 17 h, as described before. Acetone dilutions were made to dosages roughly equal to the known LD_{90}'s for unexposed, unstabilized chemicals.

Sixth instar larvae (60–120 mg) were reared on an artificial diet (Lyon et al., 1972) and the order of treatment was randomized. Chemicals were applied topically to CO_2-anesthetized larvae (1 μL/100 mg of insect weight; Robertson et al., 1975) in four replicates, with 100 insects treated per cell. Mortality counts were made after 5 days, and results were tabulated in 2 × 2 × 2 contingency tables (alive vs. dead, exposed vs. unexposed, and stabilized vs.

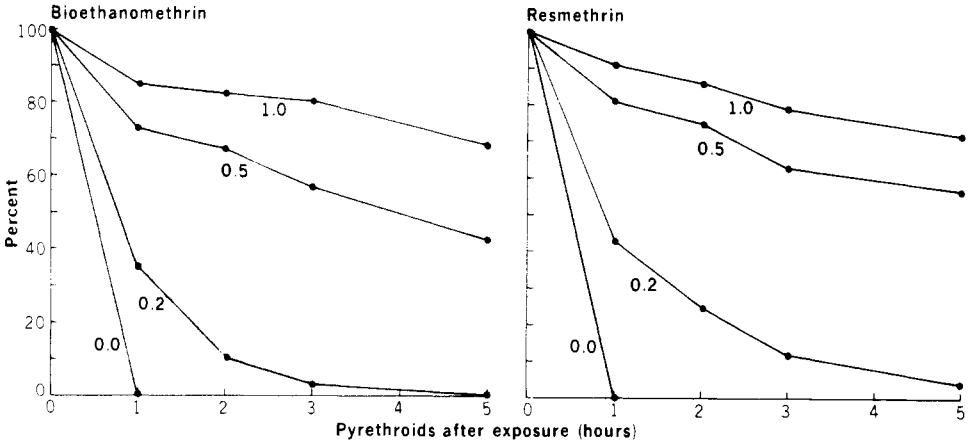

Figure 1. Percent pyrethroid remaining after sunlamp exposure of a 0.5% pyrethroid solution in Panasol AN2 with varying concentrations of Wing-Stay 100 (average of three replications).

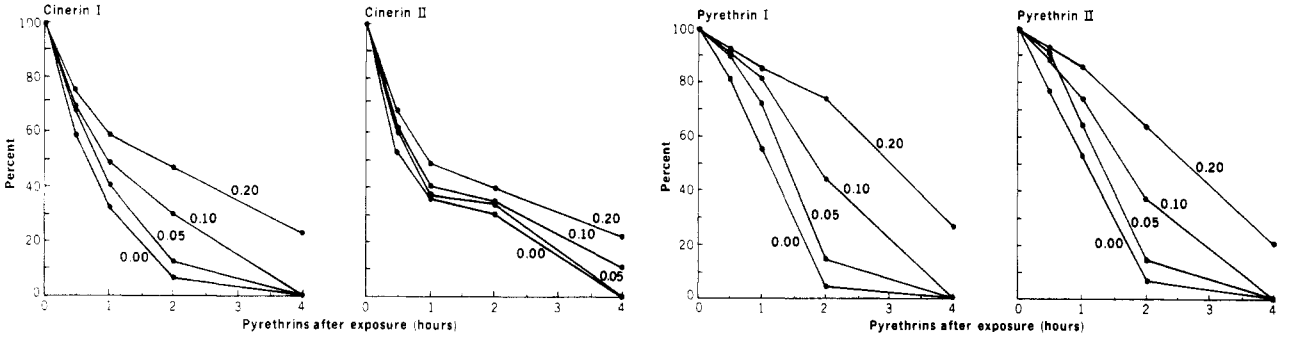

Figure 2. Percent pyrethrin remaining after sunlamp exposure of a 0.4% pyrethrum solution in Panasol AN2 with varying concentrations of Wing-Stay 100 (average of three replications).

Table I. Percent[a] Pyrethrins Remaining in Stabilized and Unstabilized Pyrethrum Formulations after Exposure to Light

formulation	cinerin I	pyrethrin I	cinerin II	pyrethrin II
sunlamp, 10 h				
F7076	57.83 ± 2.40	70.27 ± 3.35	43.86 ± 8.32	48.00 ± 8.91
PYR and WS in PAN	82.10 ± 6.35	84.73 ± 1.86	73.93 ± 8.26	80.53 ± 9.84
F7076 without stabilizers	48.00 ± 3.93	46.90 ± 9.15	31.50 ± 2.48	15.23 ± 7.25
PYR in PAN	28.20 ± 7.62	23.00 ± 11.68	41.87 ± 3.75	18.50 ± 12.20
sunlamp, 17 h				
F7076	48.33 ± 15.41	44.80 ± 17.69	32.23 ± 16.77	15.70 ± 10.38
PYR and WS in PAN	78.17 ± 4.29	84.60 ± 1.13	71.47 ± 3.37	69.67 ± 6.86
F7076 without stabilizers	28.37 ± 1.68	15.97 ± 4.05	8.90 ± 3.47	3.37 ± 3.01
PYR in PAN	9.83 ± 1.46	3.83 ± 0.83	23.20 ± 6.15	4.90 ± 0.80
sunlight, 10 h				
F7076	48.70 ± 2.82	52.47 ± 3.01	30.90 ± 2.95	23.40 ± 3.18
PYR and WS in PAN	79.53 ± 4.30	78.83 ± 2.51	61.17 ± 5.71	64.63 ± 6.19
F7076 without stabilizers	37.40 ± 2.49	39.48 ± 1.80	20.20 ± 1.91	9.37 ± 1.22
PYR in PAN	8.40 ± 2.10	4.53 ± 1.18	29.10 ± 1.59	5.73 ± 1.32

[a] Average of three replications ± standard deviation.

Table II. Percent Mortality of *C. occidentalis* Larvae Treated with Stabilized and Unstabilized Formulations, with and without Sunlamp Exposure (400 Insects per Cell)

	BEM + WS	BEM	RES + WS	RES	PYR + WS in PAN	PYR in PAN	PYR F7076	PYR F7076 minus stabil.
unexposed	69.0	65.3	65.8	57.0	90.0	90.5	90.8	88.5
exposed	35.5	2.0	50.0	1.5	65.8	2.3	46.5	15.3

unstabilized). Independence of cells was tested by a test of proportions (Lehman, 1975).

RESULTS AND DISCUSSION

Chemical Analysis. After irradiation, the estimated values of percent BEM remaining with the solvents screened by the TLC method ranged from 0 to 30% (with PAN). By use of PAN as a solvent in formulations with the antioxidants tested, the percent BEM remaining ranged from 30 to 95% (with WS). Little or no protection was observed with UV absorbers, alone or in combination with WS. A similar stabilizing effect with WS in PAN could be shown for RES. In TLC tests, protection appeared to level off at 2% WS.

The TLC findings were confirmed by data obtained by exposure of BEM and RES on glass surfaces and analysis by HPLC. The degradation of the pyrethroids under the sunlamp increased with exposure, and their protection increased with the amount of WS (Figure 1). Unprotected pyrethroids were totally destroyed within 1 h of exposure,

but with 1% WS about 70% of BEM and RES remained after 5 h of exposure.

The temperature at 30 cm from the sunlamp was approximately 70 °C. Loss due to heat-induced evaporation rather than photodegradation was estimated by placing samples in a drying oven at 70 °C for 5 h. The estimated losses were 0% for BEM and 5% for RES.

PYR formulated with PAN and WS to 0.2% showed a similar but somewhat weaker photostabilization of the four major active constituents ("pyrethrins") (Figure 2). About 10% of cinerin I and 5% of cinerin II and pyrethrins I and II were lost when PYR was exposed to 70 °C (oven) for 2 h. This indicates that some of the losses observed under sunlamp exposure were due to evaporation rather than to photodegradation. PYR formulated with WS and PAN, exposed to the sunlamp and sunlight in small Petri dishes, showed greater photostability for all major pyrethrins than the patented formulation F7076 (Table I). Without antioxidants or UV absorber, cinerin I and pyrethrin I were more stable in mineral oil, but cinerin II was more stable in PAN, while the persistence of pyrethrin II was variable.

Bioassays. The tests of proportions ($\alpha = 0.05$) showed that for all formulations (1) unstabilized, exposed chemicals killed significantly fewer insects than unexposed chemicals and (2) stabilized, exposed chemicals killed significantly more insects than unstabilized exposed chemicals (Table II).

There was no significant difference between the two unexposed, stabilized formulations. After exposure, however, the WS-stabilized formulation caused significantly greater kill ($P < 0.00006$) than F7076. A comparison of the two unstabilized pyrethrum formulations before and after exposure showed that, while there was no difference in unexposed treatments, the exposed, unstabilized F7076 clearly killed more insects than did pyrethrum in PAN ($P < 0.00006$). This finding supports the general belief that mineral oil itself, the carrier solvent in F7076, stabilizes the insecticidally active components of pyrethrum.

LITERATURE CITED

Donegan, L.; Goodin, P. J.; Thain, E. M. *Chem. Ind. (London)* **1962**, *3*, 1420.
Elliott, M.; Janes, N. F.; Potter, C. *Annu. Rev. Entomol.* **1978**, *23*, 443.
Head, S. W. *Pyrethrum Post* **1966**, *8*, 3.
Lehman, E. L. "Nonparametrics: Statistical Methods Based on Ranks"; Holden-Day: San Francisco, 1975; p 217.
LeRosen, A. L.; Moravek, R. T.; Carlton, J. K. *Anal. Chem.* **1952**, *24*, 1335.
Lyon, R. L.; Richmond, C. E.; Robertson, J. L.; Lucas, B. A. *Can. Entomol.* **1972**, *104*, 417.
Miskus, R. P.; Andrews, T. L.; Blair, P. D. U.S. Patent 3 560 613, 1971.
Robertson, J. L.; Gillette, N. L.; Look, M.; Lucas, B. A.; Lyon, R. L. *J. Econ. Entomol.* **1975**, *69*, 99.

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Received for review March 9, 1981. Revised manuscript received October 26, 1981. Accepted November 12, 1981.

High-Performance Liquid Chromatographic Determination of β-Exotoxin Produced by the Bacterium *Bacillus thuringiensis*

A high-performance liquid chromatography method was developed to measure the concentration of β-exotoxin, a potent insect toxin, produced by some strains of *Bacillus thuringiensis*. The procedure gave a rapid and direct measure of β-exotoxin concentration, and because the neutralized mobile phase of 0.1% trifluoroacetic acid in water was nontoxic to fly larvae, the column effluent could be bioassayed without concentration or extraction.

The endotoxin of *Bacillus thuringiensis* (BT) is well-known for its insecticidal properties and is available in a variety of commercial products. However, some strains of BT also produce one or more other toxins that are potent insecticides. The most studied and best characterized of these is a toxin designated β-exotoxin (Heimpel, 1967).

Vankova (1978) most recently reviewed the production, purification, bioassay, and identification procedures used in β-exotoxin studies. Bioassay procedures have been used to determine the relative efficiencies of various production and purification procedures and have indicated, by differing toxicological properties, that more than one heat-stable exotoxin may exist. Because bioassays often require weeks to complete and are subject to many variables, a rapid, sensitive analytical method that is both quantitative and qualitative is needed for β-exotoxin. We have developed a high-performance liquid chromatography (HPLC) method that fills these needs.

METHODS AND MATERIALS

Solutions to be analyzed were filtered through a Gelman 0.45-μm membrane filter and then injected into a Waters Associates Model 404 high-performance liquid chromatograph. The mobile phase of 0.1% trifluoroacetic acid in water flowed through the 3.9 mm × 30 cm C_{18} μBondapak reverse-phase column (Waters Associates) at the rate of 2 mL/min. The absorbance of eluting compounds was measured at 254 nm.

Fractions from the chromatograph were collected, adjusted to pH 7.0 by the addition of NaOH, and bioassayed for toxicity to immature horn flies, *Haematobia irritans* (L.). Larval medium was prepared by adding 4 parts of neutralized eluant or water to 6 parts of fresh bovine manure. Fifteen grams of this medium was placed in a 5-cm Petri dish and inoculated with 10 horn fly eggs. The