

Effect of Sunscreen and Antioxidant on the Stability of Pyrethrin Formulations

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The stability of pyrethrins in formulations containing different combinations of antioxidant and sunscreen was studied with model system experiments. The mechanism of disappearance of pyrethrins was mainly due to photodegradation, but some loss by codistillation can occur. In formulations, the presence of mineral oil and emulsifiers determined a decrease on codistillation losses. The presence of the antioxidant in formulations even at high concentrations did not affect pyrethrin photodegradation rates, but rising amounts of sunscreen determined a progressive increase on half-life times of pyrethrins. A combination of sunscreen and antioxidant at low concentrations provided an effective protection against sunlight similar to that obtained by high amounts of sunscreen alone.

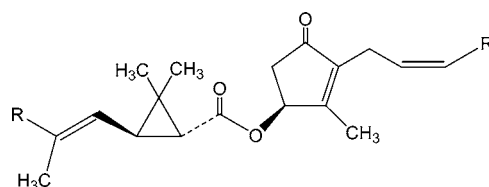
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INTRODUCTION

During the past two decades the interest in botanical insecticides has increased as a result of environmental concerns and because of insect resistance to conventional chemicals. A botanical insecticide is obtained from certain species of chrysanthemum flowers, the extract of which contains insecticidally active compounds collectively called pyrethrins (1). Pyrethrum extract is a mixture of pyrethrin I and pyrethrin II, the major insecticidal components, and another four different active ingredients, cinerin I, jasmolin I, cinerin II, and jasmolin II (Figure 1). All compounds based on chrysanthemic acid (series I) are called pyrethrins I and those based on pyrethric acid (series II), pyrethrins II. Pyrethrins II have a higher knockdown effect, whereas pyrethrins I have a higher kill effect (1).

Pyrethrins present a low impact on the environment and low mammalian toxicity and are effective on beetles, caterpillars, and various sucking insects. They are quickly degraded when exposed to air and sunlight, and this fact has limited their use in agriculture and forestry (2). As a result, commercial formulations of pyrethrins contain small amounts of antioxidants or stabilizers and synergists to improve their stability and efficacy.

Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate are antioxidants that can be used to reduce the effect of oxidation of pyrethrins. In concentrated formulations of pyrethrins, the antioxidant is present at con-



Common name	R	R'
Pyrethrins I		
cinerin I	CH ₃	CH ₃
pyrethrin I	CH ₃	CH=CH ₂
jasmolin I	CH ₃	CH ₂ CH ₃
Pyrethrins II		
cinerin II	CO ₂ CH ₃	CH ₃
pyrethrin II	CO ₂ CH ₃	CH=CH ₂
jasmolin II	CO ₂ CH ₃	CH ₂ CH ₃

Figure 1. Structures of the six pyrethrin esters.

centrations ranging from 0.01 to 2.0% (3). Synthetic synergists such as piperonyl butoxide (PBO) could be added to enhance insecticidal activity. Often a synergist is used at a 1:5 or 1:10 active ingredient/synergist ratio depending on the insect target (1) and, in the case of pyrethrins, the ratio of pyrethrins to PBO is usually 1:4 (4).

Other components such as surfactants, including emulsifiers, suspending agents, and preservatives, can be added to the composition to enhance the physical and chemical stability of the formulation (3). Miskus and Andrews (5) observed that mineral oil protects cinerin I and pyrethrin I from the effect of sunlight.

It is well-known that the main mechanism of disappearance of pyrethrins is photodegradation. In a previous work (6) the

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Table 1. Concentrations of BHT and Eusolex 6300 and Half-life Times of Pyrethrins in Pyrethrum Pale (PP) Solution and in Different Emulsions^a

	concentration (g/100 mL of final emulsion)		half-life time $t_{1/2}$ (min)				
	BHT	Eusolex 6300	cinerin II	pyrethrin II	jasmolin II	pyrethrin I	jasmolin I
PP	0	0	161a	82a	139a	73a	110a
I (control)	0	0	237bc	211b	169ab	184b	146b
II	0.035	0	259bcde	225bc	182bcd	194bcd	158bc
III	0.070	0	251bcd	232bcd	181bc	192bc	153b
IV	0	0.035	249bcd	228bc	176bc	184b	155b
V	0	0.070	279cde	240bcd	200bcd	219d	173bcd
VI	0.035	0.035	304e	301e	212cde	252e	188cd
VII	0.070	0.035	260bcde	259cde	187bcd	217cd	162bc
VIII	0.070	0.070	301e	299e	218de	257e	164bc
IX	0.035	0.070	285de	271de	200bcd	223d	169bcd
X	0.28	0	228b	217bc	168ab	194bc	145b
XI	0	0.28	305e	261cde	240e	258e	195d

^a Values within a column for each compound having different letters are significantly different from each other using Duncan's multiple-range test ($p < 0.05$).

photodegradation behavior of pyrethrins in three different commercial formulations and in pyrethrum pale (PP) was reported. Other papers reported the rapid breakdown of pyrethrins under field conditions (2, 7). To protect the active ingredients from the effect of sunlight, sunscreens should be added to the commercial formulation of this botanical insecticide (8). The aim of the present paper is to test the efficacy of a sunscreen of low toxicity to improve the stability of pyrethrins in formulations.

EXPERIMENTAL PROCEDURES

Chemicals. Pestanal technical mixture of pyrethrins, purity = 21.58% (10.62% pyrethrin I; 7.00% pyrethrin II; 1.51% cinerin I; 1.25% cinerin II; 0.70% jasmolin I; 0.50% jasmolin II) was purchased from Fluka Riedel-de-Haën (Milan, Italy). PP, a commercial concentrate extract containing 50% of pyrethrins, was kindly donated by Serbios (S. Colombano Lambro, Italy). Sorbitan monooleate (Span 80), polyoxyethylene sorbitan monooleate (Tween 80), and BHT were purchased from Sigma Aldrich (Milan, Italy). 4-Methylbenzylidene camphor (Eusolex 6300) was from Merck (Darmstadt, Germany). Mineral oil was from Galeno (Comeana, Italy). Hydrophilic Teflon (PTFE) membrane filters (TE, 0.45 μ m) were purchased from Whatman (Maidstone, Kent, U.K.). Acetone and acetonitrile were HPLC grade solvents (Carlo Erba, Milan, Italy), whereas *n*-hexane (Carlo Erba) was a special reagent for pesticide determination. Water for HPLC analysis was obtained with a Milli-Q water purification system (Millipore, Milford, MA).

Stock standard solutions of pyrethrins (~1000 mg/kg) were prepared in *n*-hexane or acetone. Suitable volumes of stock solution were evaporated to dryness, and residues were dissolved in appropriate volumes of an acetonitrile/water solution (50:50, v/v) to obtain working standard solutions.

Apparatus and Chromatography: HPLC Analysis. An Agilent Technologies (Waldbronn, Germany) model 1100 liquid chromatograph fitted with a diode array detector (DAD), UV 6000 LP (Thermo Quest, San Jose, CA), was used. A Waters X Terra RP₁₈ (250 \times 4.6 mm, 5 μ m) column was employed. For the separation of pyrethrins was used a gradient as follows: initial mobile phase acetonitrile/water (50:50; v/v), hold for 3 min, reaching acetonitrile/water 80:20 (v/v) in 15 min, hold for 5 min, and reconditioned for 8 min with the initial mobile phase. The injection volume was 50 μ L, and the flow rate was 1 mL/min. The analysis was performed at a wavelength of 230 nm according to the maximum reported in the UV spectrum.

Preparation of Oil/Water (O/W) Emulsions. Eleven different emulsions were studied. To obtain the emulsions, two solutions were prepared. Solution A was prepared by weighing in a glass vial mineral oil, PP, Span 80, and, if present in the formulation, BHT and Eusolex 6300. Solution B was prepared by mixing in a glass vial water and Tween 80. Appropriate amounts of solutions A and B were mixed together to give a final pyrethrins concentration of 0.28% (w/v). The

mixture was emulsified to produce an O/W emulsion with a rotor/stator homogenizer T 25 Ultra Turrax (Janke und Kunkel GmbH and Co KG, Staufen, Germany) for 1 min at 8000 rpm. The emulsion was further homogenized to produce a fine O/W emulsion with small oil droplets using a high-pressure homogenizer Emulsiflex-C5 (Avestin, Ottawa, Canada) at a pressure of 250 bar for three cycles.

A basic emulsion I (control) was prepared without BHT and Eusolex 6300 at the following concentrations (g/100 mL of final emulsion): mineral oil (4.76); Span 80 (0.46); Tween 80 (0.54); and pyrethrum extract (0.56). Emulsions II–XI were prepared by adding to the basic emulsion different amounts of BHT and Eusolex 6300. The concentrations of BHT and Eusolex 6300 for each emulsion are reported in Table 1.

The content of pyrethrins in the emulsions was the same used in field trials with commercial formulations. BHT, a food-grade antioxidant, was used to evaluate its effectiveness on pyrethrins stabilization. Formulations were prepared with increasing amounts of BHT (0.035, 0.070, and 0.28%) according to the range of concentrations recommended by Bencsits (10). Eusolex 6300, an UV-B sunscreen, was chosen according to the spectral range (290–320 nm) responsible for the photodegradation of pyrethrins (5). This sunscreen also presents low toxicity, with an LD₅₀ (oral, rat) of >16000 mg/kg (11).

Model System. Sunlight Photodegradation Experiments. The experiments of sunlight photodegradation were carried out with PP solution in acetone and with the emulsions reported in Table 1.

Twenty microliter aliquots of solution or emulsion were poured into Petri dishes and evaporated at room temperature. The dishes were exposed to direct sunlight and removed at prefixed intervals (0, 30, 90, 180, 300, and 420 min) from the sunlight. A control was kept in the dark in the laboratory. The residue contained in the dishes was dissolved with 5 mL of initial mobile phase and then injected for HPLC analysis. The experiments were carried out in three replicates. The samples were irradiated on November 17, 2004, between 9 a.m. and 4 p.m. During the trial the average daily solar radiation was 220 W/m² (solar constant = 1350 W/m²).

Evaporation, Thermodegradation, and Codistillation Experiments. The experiments were conducted according to the procedure described in a previous study (9). For these experiments solutions of PP and commercial formulation were prepared in acetone, and formulations VI and VIII were prepared in a hexane/acetone mixture (50:50, v/v) instead of water. Briefly, for evaporation and thermodegradation experiments, Teflon membrane filters added with each prepared solution were placed inside amber vials with a screw-closed cap. After the vial had been kept in the oven at 50 °C for 24 h, the residues in the filter and on the walls were extracted and analyzed by HPLC. Control filters were kept in the dark at room temperature.

For codistillation experiments, membrane filters treated as previously described were placed on the top of vials containing 5 mL of water with a screw cap with a center hole. The procedure was the same as described above for the evaporation experiments. All experiments were conducted in three replicates.

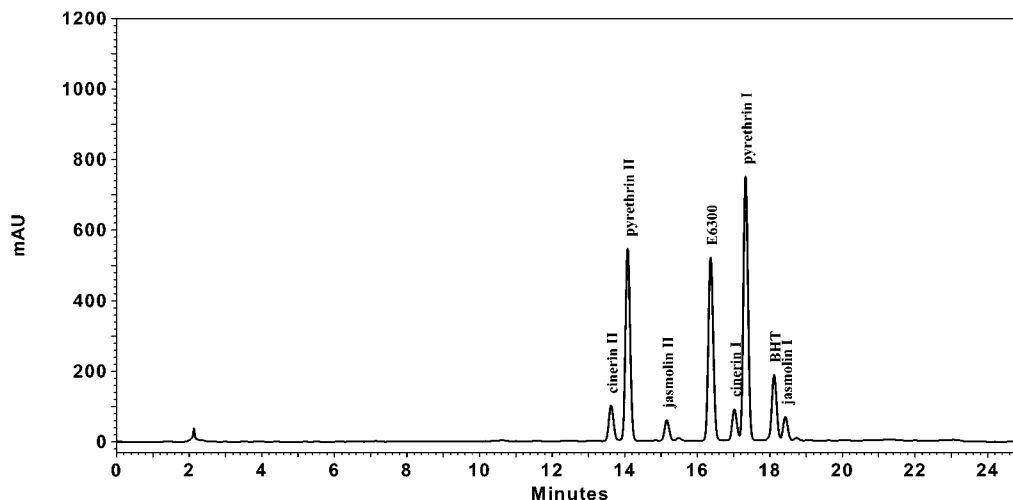


Figure 2. Chromatogram of pyrethrins in a formulation with BHT and Eusolex 6300 using the HPLC method described.

Comparison between the amount of residue on the filter and that present on the control filter shows the amount of active ingredient that codistills. To obtain a correct evaluation of these data, we have to consider the amount of residue lost by evaporation and by thermodegradation.

Statistical Analysis. Mean comparisons were performed by Student's *t* test at $p < 0.05$, when appropriate. Analysis of variance was performed by GenStat, 7th ed. (2003), when appropriated ($p < 0.05$); analysis was followed by the Duncan post hoc test.

RESULTS AND DISCUSSION

HPLC Analysis. The HPLC conditions reported before allowed a good separation of the six pyrethrin esters, antioxidant, BHT, and sunscreen, Eusolex 6300 (**Figure 2**). A good linearity was obtained in the range of 0.03–14 mg/kg for total pyrethrins with correlation coefficients between 0.9996 and 0.9999 for each ester.

Model System. Sunlight Photodegradation Experiments. A preliminary study was carried out with formulations at different ratios of pyrethrins/mineral oil (1:1, 1:5, 1:10, and 1:17), without BHT or Eusolex 6300, to evaluate the effect of mineral oil on pyrethrin photodegradation. As reported by Crosby (8), mineral oil could afford some protection against light. Actually, we observed that degradation rates of pyrethrins I and II in formulations were lower than those obtained for the pyrethrum pale solution. The decay rates were slightly affected, increasing the content of mineral oil in the formulation; thus, the ratio 1:17 used in formulations was chosen exclusively for technical reasons.

Decay rates were calculated as a pseudo-first-order kinetics (correlation coefficients between 0.901 and 0.995). **Table 1** reports half-life times of pyrethrin esters in PP solution and formulations obtained from three replicates with a maximum coefficient of variation (CV) of 15%. The data for cinerin I were not calculated due to overlapping of the peak of the metabolite of pyrethrin I. Data reported in **Table 1** for formulation I (control) show that the presence of additives in the formulation provides a significant decrease in photodegradation rates when compared to the pyrethrum pale solution.

Half-life times calculated for pyrethrins in formulations II, III, and X showed that the presence of BHT alone at different concentrations did not affect the stability of pyrethrins if compared to control. This indicates that this antioxidant does not protect pyrethrins from photodegradation and that the decrease in half-life times is due only to the presence of mineral oil.

The opposite effect was observed for formulations prepared with sunscreen alone. Experiments carried out with formulations V and XI showed that rising amounts of Eusolex 6300 determined a progressive increase in the half-life times of pyrethrin I, whereas for formulation IV, containing Eusolex 6300 at 0.035%, the changes in photodegradation rates were not significant.

The combination of sunscreen and antioxidant at a 1:1 ratio provided a remarkable stabilization of pyrethrins to photodegradation. Half-life time values calculated for both formulations VI and VIII were quite similar to those obtained with formulation XI, and when considered the values for pyrethrin II, they were 15% higher.

Formulations VII and IX, at the other ratios (1:2 and 2:1), showed values of decay rates that did not exceed those found for 1:1 ratio. These data indicated that higher amounts of Eusolex 6300 and/or BHT do not afford an increase in pyrethrin half-life times.

Evaporation, Thermodegradation, and Codistillation Experiments. Pyrethrins did not show any evaporation or thermodegradation because no residues were found on the walls of the vials and no significant difference (<4% for pyrethrin I) was observed between residues in membrane filters placed in the oven and in control membrane filters.

The data obtained for codistillation experiments are reported in **Table 2**. Experiments carried out with the pyrethrum pale solution showed that pyrethrin esters have a tendency to codistillate and, as predicted by Henry's law constant, the pyrethrins of the I series volatilize from water rather more rapidly than those of the II series (7). Forty-two percent of pyrethrin I was lost when the experiment was performed with PP, and only 16% was lost when the commercial formulation was used. The same effect was observed for other esters that showed reductions in codistillation losses ranging from 50 to 100%.

When the experiments were performed with formulations VI and VIII, codistillation losses of pyrethrin II, cinerin I, and pyrethrin I were observed. Pyrethrin II losses in both formulations (~20%) were similar to that obtained for PP (17%). This indicates that pyrethrin II codistillation is not affected by the presence of the components of the formulation.

An inverse behavior was observed for cinerin I and pyrethrin I. Formulation VI provided codistillation data similar to those observed for PP with 35% losses for both esters, but for formulation VIII, when the amounts of BHT and Eusolex 6300

Table 2. Determination of the Effect of Codistillation on Pyrethrins (Milligrams per Kilogram \pm SD) in the Pyrethrum Extract, Commercial Formulation, and Prepared Formulations VI and VIII

	codistillation			%
	control (k)	filter (m)	diff [k - m]	
pyrethrum extract				
cinerin II	0.96 \pm 0.05	0.85 \pm 0.06	ns ^a	
pyrethrin II	5.41 \pm 0.31	4.49 \pm 0.25	0.92	17
jasmolin II	0.56 \pm 0.03	0.49 \pm 0.03	ns	
cinerin I	0.71 \pm 0.05	0.33 \pm 0.03	0.38	54
pyrethrin I	6.52 \pm 0.46	3.79 \pm 0.29	2.73	42
jasmolin I	0.67 \pm 0.04	0.49 \pm 0.06	0.18	26
commercial formulation				
cinerin II	0.90 \pm 0.08	0.86 \pm 0.03	ns	
pyrethrin II	4.07 \pm 0.31	4.13 \pm 0.19	ns	
jasmolin II	0.36 \pm 0.02	0.34 \pm 0.03	ns	
cinerin I	0.64 \pm 0.04	0.50 \pm 0.08	0.14	22
pyrethrin I	4.35 \pm 0.24	3.66 \pm 0.42	0.69	16
jasmolin I	0.53 \pm 0.09	0.49 \pm 0.06	ns	
formulation VI				
cinerin II	0.74 \pm 0.03	0.69 \pm 0.05	ns	
pyrethrin II	4.20 \pm 0.11	3.26 \pm 0.12	0.94	22
jasmolin II	0.37 \pm 0.02	0.34 \pm 0.02	ns	
cinerin I	0.70 \pm 0.03	0.46 \pm 0.06	0.24	35
pyrethrin I	6.40 \pm 0.33	4.19 \pm 0.44	2.21	35
jasmolin I	0.61 \pm 0.05	0.57 \pm 0.05	ns	
formulation VIII				
cinerin II	0.67 \pm 0.02	0.66 \pm 0.02	ns	
pyrethrin II	4.48 \pm 0.11	3.59 \pm 0.19	0.88	20
jasmolin II	0.35 \pm 0.01	0.35 \pm 0.00	ns	
cinerin I	0.69 \pm 0.01	0.57 \pm 0.02	0.12	18
pyrethrin I	6.32 \pm 0.14	5.10 \pm 0.31	1.23	19
jasmolin I	0.57 \pm 0.01	0.58 \pm 0.02	ns	

^a Not significant.

used were 2 times higher, the losses were 18 and 19%, respectively.

From the data reported for both formulations (VI and VIII) in the experiments of the model system, we point out that the most effective stabilization of pyrethrins is achieved when formulation VIII is used.

Conclusions. Data from the model system for evaporation, thermodegradation, and codistillation experiments showed that pyrethrins do not have a tendency to evaporate or thermodegrade, but some loss by codistillation can occur. The presence of mineral oil and emulsifiers in the formulation determined a significant decrease on codistillation losses of pyrethrins I.

The data obtained from photodegradation experiments indicated that the use of sunscreen in formulations improves the effectiveness of pyrethrins stabilization, but a similar effect is not achieved by using the antioxidant alone. A combination of sunscreen and antioxidant at low concentrations afforded the same protection against photodegradation that can be provided by high amounts of sunscreen alone. As predicted by Crosby (8), an effective reduction in the photodegradation of pyrethrins can be achieved by incorporation of antioxidants and sunscreens into the formulations.

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