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Degradation of Pyrethrin Residues on Stored Durum Wheat after Postharvest Treatment

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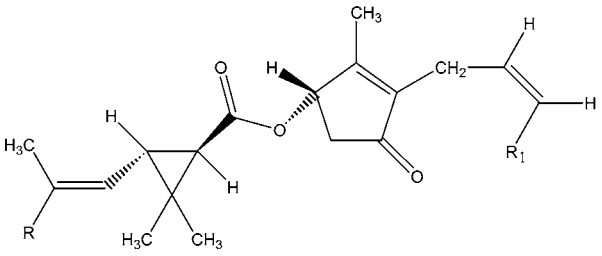
In this paper, pyrethrin levels during a postharvest treatment on stored durum wheat were studied. Two experiments were carried out at single and double the dose recommended by the manufacturer. In all trials, the initial deposition of pyrethrins levels was below the fixed maximum residue level of 3 mg/kg. The fate of pyrethrins in the two experiments was similar, and the total content of pyrethrins remained unchanged for 22 days with a complete dissipation in 8 months. In the single dose experiment, half-life times of pyrethrins I and II were 46 and 72 days, while for the double dose, pyrethrins I and II were 41 and 53 days, respectively.

KEYWORDS: Pyrethrins; stored wheat; LC-MS; HPLC-DAD

INTRODUCTION

In the last years, especially in western countries, organic foods are becoming much more widely available. Organic agriculture only uses naturally occurring chemicals or traditional remedies to control pests and diseases. On the other hand, processed organic foods usually contain only, or at least a specified percentage of organic ingredients and no artificial food additives, no pesticide residues, and are often processed with fewer artificial methods, materials, and conditions (e.g., no chemical ripening and no food irradiation). Among them, organic bread and bakery products are prepared using organically grown whole wheat. Grains are frequently stored long term (3−36 months) at ambient temperature in bulk silos where insecticides may be applied postharvest to reduce losses from storage pests. In Italy, naturally occurring pyrethrins are a registered product for stored wheat with a maximum residue level (MRL) of 3 mg/kg (*1*). Pyrethrum extract is a botanical nonsystemic insecticide with contact action commonly used with the synergist piperonyl butoxide (*2*). Pyrethrum contains three esters of chrysanthemic acid (pyrethrins I) and three corresponding esters of pyrethric acid (pyrethrins II) (**Figure 1**) that are insecticidally active and extracted from the plant *Chrysanthemum cinerariaefolium* (*3*). Pyrethrin I and pyrethrin II are the most prominent of the six different active ingredients. The other four different active ingredients are cinerins I and II and jasmolins I and II. The natural mixture of esters has been used safely for the past 160 years as a botanical insecticide around the world (*4*). Pyrethrins are fast acting and toxic to insects at low doses, but they are not considered a satisfactory insecticide against agricultural pests due to instability toward heat, light, and air and also for the tendency for insects to recover from sublethal doses. They are widely used as home and garden insecticides along with uses on pets and livestock, mosquito control, treatment of transport vehicles, and for treatment of ectoparasitic disease.



R = -CH_3 (chrysanthemates) or -CO_2CH_3 (pyrethrates)

R_1 = -$CH{=}CH_2$ (pyrethrin) or -CH_3 (cinerin) or -CH_2CH_3 (jasmolin)

Figure 1. Chemical structures of pyrethrins I and II.

Because of their photolability, pyrethrins when exposed to sunlight showed half-life times of a few minutes (*5*), while in a field experiment on peaches, half-life times were in the order of days (*6*). It is also reported that in storage tests, repellency of one part of pyrethrum synergized with 10 parts of piperonyl butoxide has been shown to continue after 9 months or more (*7*). Prolonged storage of harvested pyrethrum crop has resulted in substantial losses of pyrethrin esters due to the environment in the storage shed conditions such as the temperature at which the crop is stored. (*8*). Little is known about the fate and stability of pyrethrins on storage conditions. For this reason, we report the study of the persistence of pyrethrins in a postharvest treatment on stored durum wheat.

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Table 1. LC/MS (APCI+) Characteristics of Pyrethrins

compound	HPLC t_R (min)	mol wt	LC/MS (APCI) *m/z* (amu) (% relative abundance)
cinerin I	33.42	316.43	317 $[M + H]^+$ 100, 358 $[M + H + CH_3CN]^+$ 19
pyrethrin I	34.45	328.44	329 $[M + H]^+$ 100, 370 $[M + H + CH_3CN]^+$ 10
jasmolin I	37.39	330.46	331 $[M + H]^+$ 100, 372 $[M + H + CH_3CN]^+$ 10
cinerin II	26.40	360.44	361 $[M + H]^+$ 100, 378 $[M + H_2O]^+$ 55, 402 $[M + H + CH_3CN]^+$ 33
pyrethrin II	27.25	372.45	373 $[M + H]^+$ 100, 390 $[M + H_2O]^+$ 25, 414 $[M + H + CH_3CN]^+$ 15
jasmolin II	29.20	374.47	375 $[M + H]^+$ 100, 392 $[M + H_2O]^+$ 49, 416 $[M + H + CH_3CN]^+$ 15

MATERIALS AND METHODS

Chemicals. Pyrethrin standards (Pestanal grade at 21.58%; cinerin II, 1.25%; pyrethrin II, 7.00%; jasmolin II, 0.50%; cinerin I, 1.51%; pyrethrin I, 10.62%; and jasmolin, 0.70%) were obtained from Sigma-Aldrich (Milan, Italy). Water was distilled and filtered through a Milli-Q apparatus (Millipore, Milan, Italy). Trifluoroacetic acid, acetonitrile, and methanol were high-performance liquid chromatography (HPLC) grade solvents (Carlo Erba, Milan, Italy). Hexane was of gas chromatography grade (Carlo Erba). Magnesium sulfate was analytical grade (Carlo Erba). PTFE syringe filters of 0.45 μm were from PALL Life Sciences (Ann Arbor, MI).

A stock standard solution of the active ingredient was prepared in hexane. Working standard solutions for HPLC analyses were prepared daily from the stock solution by evaporating the solvent and dissolving the residue with the mobile phase (water/acetonitrile, 50:50 v/v).

Storage Experiments. The experiments were carried out following a scheme design with four replications. Each test sample of 100 kg of stored wheat (*Triticum durum*) was treated with the commercial formulation obtained from COPYR (Tradate, Italy) at 2% of active ingredient (a.i.) at the dose recommended by the manufacturer and at double dose. An aliquot of the commercial formulation (6.1 g of formulated product/100 kg/grain) was diluted with water 6.25 and 12.5 times, respectively, and applied to grains. Treatment was carried out using 82.5 mL/test sample. The compound was sprayed with a manual sprayer (GDM professional, Italy). Treatment was carried out on the proper amount of wheat distributed on top of a plastic sheet of 36 square meters on October 24, 2005. After 30 min from the treatment, the test sample was homogenically placed in hermetically closed plastic bins of 100 kg capacity. The control was carried out treating wheat using 82.5 mL of water.

Samplings, on dry wheat, started about 1 h after treatment; random 2 kg samples of wheat were collected from each plot in dark plastic bags and immediately analyzed for pyrethrin residues. The sampling and the analysis were repeated at 0, 1, 4, 7, 9, 14, 22, 80, 120, and 240 days after treatment (DAT).

Wheat sampling was carried out using a hand-probe of aluminum with a length of 1.2 m. The probe consisted of two tubes, one inside the other. The grain probe was 15 cm in diameter (outer tube). The inner tube was divided into compartments. The outer tube had slots, which matched the compartment openings of the inner tube. When the tubes were aligned, grains may enter into or be emptied from the compartments of the probe. During the trial, the maximum and minimum temperatures were 28 and 8 °C, respectively.

Apparatus and Chromatography. *HPLC−Diode Array Detection (DAD) Analysis*. An Agilent Technologies (Waldbronn, Germany) model 1100 liquid chromatograph fitted with a DAD, UV 6000 LP (Thermo Quest, San Jose, CA), was used. A Waters X Terra RP18 (250 mm × 4.6 mm, 5 μm) column was used. Separation of pyrethrins was achieved using the following gradient: 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 concentration. The injection volume was 50 μL, and the flow rate was 1 mL/min. The analysis was performed at 230 nm wavelength according to pyrethrin maximum absorbances reported in the UV spectrum and ranging from 227 to 235 nm.

LC-MS Analysis. An HPLC system (Shimadzu, Milan, Italy) equipped with an SPD11 Avp DAD detector, an SIL 11 AD vp autoinjector, and a LC 10 AD binary pump coupled on line with an MS2010 mass spectrometer (Shimadzu) was used. UV and MS data were acquired and processed using Shimadzu "LCMS solution" software. Gradient development was with acetonitrile−aqueous 0.1% trifluoroacetic acid 99% (30/70, v/v) at 0 min to acetonitrile−aqueous 0.1% trifluoroacetic acid 99% (0:100, v/v) at 40 min. The column used was a 150 × 2.1 mm i.d. 3.5 μm Waters Symmetry C18. The injection volume was 20 μL, and the flow rate was 0.2 mL/min. MS conditions were as follows: APCI(+) source probe, 350 °C; CDL, 300 °C; block at 290 °C; flow gas (N_2) at 2.5 mL/min; probe voltage, 4.5 kV; and scan, 200−500 amu. The mass detector was operated in the single ion monitoring mode acquiring the most abundant LC-MS adducts for each pyrethrin (**Table 1**).

Extraction Procedure from Wheat. Four samples were taken per DAT. A 10 g aliquot of wheat grain was weighed in a 40 mL screw-capped tube; 0.2 g of magnesium sulfate and 10 mL of acetonitrile were added. The tube was agitated for 20 min in a rotatory shaker at 9 rpm and then stored at −20 °C for 30 min. The liquid phase was filtered with PTFE syringe filters, and 2 mL was evaporated to dryness under a gentle nitrogen stream. The residue was dissolved with 400 μL of the mobile phase (water/acetonitrile, 50:50 v/v) and sonicated for 30 s. The solution was then centrifuged at 4500 rpm for 5 min, and the supernatant (50 μL) was injected for HPLC analysis. The amount of sample in the final extract was 5.00 g/mL.

Recovery Studies. Samples of untreated wheat grain were fortified with the appropriate aliquot of working standard solution of pyrethrins to reach concentrations of 0.10, 0.2, 0.5, 1.0, and 2.0 mg/kg as total pyrethrins. Prior to the extraction step, the fortified samples were allowed to settle for 30 min and then processed according to the above procedure. Three replicates for each concentration were analyzed.

RESULTS AND DISCUSSION

HPLC-DAD and LC-MS Analysis. Because pyrethrins have strong UV absorption at 230 nm, a reversed phase HPLC method was developed using DAD for detection of pyrethrin residues on wheat. An HPLC-DAD chromatogram of a standard of pyrethrins together with the blank is reported in **Figure 2**. HPLC retention times for pyrethrins were as follows (in min): cinerin II, 14.04; pyrethrin II, 14.50; jasmolin II, 15.61; cinerin I, 17.47; pyrethrin I, 17.78; and jasmolin I, 18.90. No interfering peaks were detected at the retention times of all analytes. Analysis of pyrethrins formulation was conducted by diluting 10 mg of the commercially available formulation with 10 mL of methanol followed by a dilution with the mobile phase acetonitrile/water (50:50; v/v) and using the same HPLC-DAD chromatographic method.

We applied a previously reported liquid chromatographic mass spectrometric method using the atmospheric pressure chemical ionization technique (LC-APCI-MS) in order to ensure accurate identification and confirmation of the selected pyrethrins (*7*). **Table 1** reports LC-MS-APCI(+) fragmentation pattern of pyrethrins with the most abundant ion adduct $[M + H]^+$ for all pyrethrins.

Calibration graphs for the active ingredients were plotted reporting peak height vs concentration. A good linearity was obtained in the range from 0.10 to 10 mg/kg for total pyrethrins with correlation coefficients between 0.9996 and 0.9999.

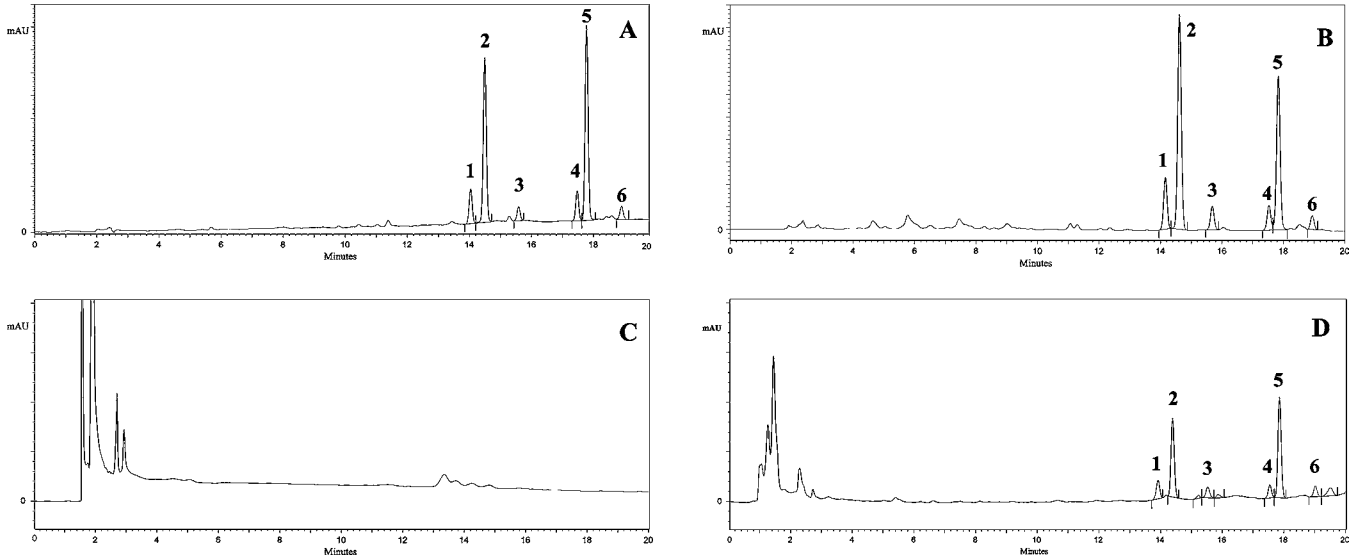

Figure 2. Chromatograms at 230 nm for the analysis of pyrethrins: 1, cinerin II; 2, pyrethrin II; 3, jasmolin II; 4, cinerin I; 5, pyrethrin I; and 6, jasmolin I. (**A**) A standard of pyrethrins at 1 mg/kg, (**B**) commercial formulation, (**C**) a blank of wheat, and (**D**) a blank of wheat fortified at 0.70 mg/kg of total pyrethrins.

Table 2. Average Pyrethrin Residues (mg/kg, $n = 4$) in Durum Wheat After 1 and 2× Treatment at the Manufacturer's Recommended Dose

time (days)	cinerin II	pyrethrin II	jasmolin II	cinerin I	pyrethrin I	jasmolin I	totals
			single dose				
0	0.06 ± 0.02	0.36 ± 0.05	ND[a]	0.06 ± 0.02	0.53 ± 0.07	0.05 ± 0.01	1.06
1	0.07 ± 0.01	0.34 ± 0.06	ND	0.07 ± 0.02	0.47 ± 0.09	0.05 ± 0.01	1.00
4	0.08 ± 0.02	0.38 ± 0.08	ND	0.08 ± 0.02	0.60 ± 0.14	0.06 ± 0.02	1.20
7	0.08 ± 0.01	0.36 ± 0.02	ND	0.08 ± 0.01	0.58 ± 0.04	0.06 ± 0.01	1.16
9	0.08 ± 0.01	0.37 ± 0.10	ND	0.06 ± 0.02	0.55 ± 0.13	0.06 ± 0.02	1.12
14	0.05 ± 0.02	0.34 ± 0.12	0.05 ± 0.02	0.05 ± 0.02	0.48 ± 0.18	0.07 ± 0.06	1.04
22	0.08 ± 0.02	0.35 ± 0.04	0.05 ± 0.01	0.06 ± 0.02	0.52 ± 0.08	ND	1.06
80	0.04 ± 0.02	0.17 ± 0.02	ND	0.02 ± 0.02	0.12 ± 0.02	ND	0.35
120	0.02 ± 0.01	0.09 ± 0.03	ND	ND	0.06 ± 0.02	ND	0.17
240	ND	ND	ND	ND	ND	ND	ND
			double dose				
0	0.14 ± 0.02	0.66 ± 0.13	0.07 ± 0.01	0.13 ± 0.04	1.06 ± 0.22	0.12 ± 0.02	2.18
1	0.16 ± 0.04	0.68 ± 0.08	0.08 ± 0.02	0.16 ± 0.04	1.17 ± 0.13	0.09 ± 0.02	2.34
4	0.16 ± 0.04	0.84 ± 0.11	0.09 ± 0.02	0.16 ± 0.02	1.32 ± 0.17	0.13 ± 0.02	2.70
7	0.16 ± 0.04	0.86 ± 0.10	0.09 ± 0.02	0.16 ± 0.04	1.33 ± 0.17	0.14 ± 0.04	2.74
9	0.15 ± 0.06	0.82 ± 0.14	0.09 ± 0.02	0.17 ± 0.06	1.29 ± 0.20	0.14 ± 0.04	2.66
14	0.14 ± 0.02	0.71 ± 0.09	0.08 ± 0.01	0.12 ± 0.04	1.07 ± 0.12	0.14 ± 0.02	2.26
22	0.21 ± 0.04	0.82 ± 0.10	0.12 ± 0.03	0.14 ± 0.04	1.21 ± 0.15	0.12 ± 0.02	2.62
80	0.02 ± 0.01	0.42 ± 0.08	0.06 ± 0.01	ND	0.52 ± 0.12	0.06 ± 0.02	1.08
120	ND	0.25 ± 0.05	ND	0.03 ± 0.01	0.23 ± 0.03	ND	0.51
240	ND	0.03 ± 0.01	ND	ND	0.02 ± 0.01	ND	0.05

[a] ND, not detectable.

HPLC-DAD limits of quantification (S/N = 10) were 0.01, 0.02, and 0.05 mg/kg for pyrethrins, cinerins, and jasmolins, respectively. Mean recoveries for wheat ranged from 86 to 111% with coefficients of variation between 4 and 12%. Both the precision under conditions of repeatability and the intermediate precision were determined by performing either six injections of 0.15 and 0.75 mg/L as the sum of pyrethrins in the same day or six injections of the same standards in different days, respectively. The highest and the lowest coefficients of variation were 12.5 and 0.4% for repeatability except for jasmolin II, which was not detected at the 0.15 mg/L level of pyrethrins, 11.9 and 1.1% for intermediate precision, respectively.

Residues. The formulation used had a lower content of pyrethrin I if compared with pyrethrin II (31.4 and 45.6%, respectively), while concentrations of cinerin I and II were 4.2 and 10.8%, respectively, and the concentrations of jasmolin I and II were 2.9 and 5.1%, respectively. The initial deposit of total pyrethrins after the two dose treatment was in accordance with the calculated values of 1.31 and 2.62 mg/kg, respectively, and for the individual active principles if compared with percentages obtained with the chromatographic analysis of the commercial formulation (**Table 2**). For both experiments, the total content of pyrethrins did not change for 22 days and then decreased progressively until complete disappearance after 8 months. The decline of pyrethrins residue in storage and processing can be explained in terms of the physicochemical properties of the active ingredients and the nature of the process. Residues of the lipophilic pyrethrins tend to remain on the seed coat although a proportion can migrate through to the bran and germ, which contain high levels of triglyceride. Storage fungi may also assist in the degradation of the insecticide (*10*). Pyrethrins half-life times were calculated as a pseudo first-order kinetic with correlation coefficients greater than 0.976. In the experiment using a single dose, the half-life times of pyrethrins

I and II were 46 and 72 days, while for the double dose pyrethrins I and II were 41 and 53 days, respectively. The dissipation trend of pyrethrins for the two experiments was similar.

With the experiment at the dose recommended by the manufacturer at $T = 0$, the sum of pyrethrins I and II is 1.06 mg/kg, far from the Italian MRL on stored wheat. With the experiment at 2× the dose recommended at $T = 0$, the sum of pyrethrins I and II is 2.18 mg/kg, still below the Italian MRL on stored wheat.

These storage experiments, carried out in the absence of light, showed that pyrethrins are more stable if compared with field experiments where pyrethrins are quickly photodegraded (*7*). In conclusion, field persistence of pyrethrins could be ameliorated by applying formulations prepared with additives able to protect pyrethrins from photodegradation and helping the penetration rate of the a.i. in the vegetal tissues of treated crops.

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