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ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · JANUARY 2007

Impact Factor: 2.91 · DOI: 10.1021/jf062461v · Source: PubMed

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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Residues and Persistence of Neem Formulations on Strawberry after Field Treatment

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Azadirachtoids were determined by liquid chromatography/mass spectrometry (LC/MS) in five methanolic seed extracts of the neem tree and in a commercial formulation. On average, seed extracts contain azadirachtin A (10.9%), azadirachtin B (3.5%), nimbin (10.4%), and large quantities of salannin (19.0%). The composition of the commercial formulations may present different azadirachtoids contents depending on the natural extracts used in the preparation. Because these compounds may also show insecticide activity, the efficacy on field of these formulations may be very different. Photodegradation of pure azadirachtoids was also studied. Azadirachtins and related compounds are very sensitive to sunlight, degrading rapidly, with half-lives of the order of 11.3 h for azadirachtin A and 5.5 h for azadirachtin B and few minutes for the other limonoids compounds studied. The residues of azadirachtins and the main constituents, e.g., salannin, nimbin, deacetylnimbin, and deacetylsalannin, of the neem seed extract were determined on strawberries after field treatment using two different formulations. This residue study on strawberry was carried out to assess not only the azadirachtin content but also the main azadirachtoids contents. Three days after field application at five times the dose recommended by the manufacturer, residues of azadirachtin A and B were 0.03 and 0.01 mg/kg, respectively, while residues of salannin (LOQ 0.01 mg/kg) and nimbin (LOQ 0.5 mg/kg) were not detectable.

KEYWORDS: HPLC/DAD; LC-ESI-MS; azadirachtin A; azadirachtin B; deacetylnimbin; deacetylsalannin; nimbin; salannin; neem oil; strawberry; field trial

INTRODUCTION

Azadirachtin A (AZA-A) is the biologically active limonoid of the tetranortriterpenoid type extracted from seeds of the neem tree (*Azadirachta indica*) native to Southeast Asia but growing in many countries throughout the world. Neem seed extracts also contain other biologically active chemical compounds, namely, azadirachtin B (AZA-B), deacetylnimbin, deacetylsalannin, nimbin, and salannin (*1*−*3*). Chemical structures of the limonoids of interest are reported **Figure 1**. Other tissues of *Azadirachta indica* known to contain these compounds at lower levels are the bark, leaves, and heartwood (*4*). Because seeds contain the highest concentrations of biologically active compounds, most experimental and commercial preparations of neem are represented by their extracts (*1*). Aqueous, methanolic, and ethanolic extracts of neem seeds show biological activity in the laboratory and in the field, although at a varying extent to different target organisms (*4*).

The biological activity of azadirachtin and related compounds is thought to be derived by successive rearrangement and oxidation products of tirucallol (*4*). Within the azadirachtin molecule, the decalin fragment is responsible for the insect growth regulation and development effect observed, while the hydroxyl furan fragment causes the antifeedant effect among target species (*5*). Other compounds present in neem seed extracts, like salannin and nimbin, may exhibit oviposition repellency, egg sterility, longevity, fitness, and inhibition of chitin biosynthesis (*4*). The use of a commercial formulation of neem successfully deterred aphids attempting to land, probe, or oviposit (*6*). Isman et al. suggest that this deterrence effect results from different compounds working synergically and producing different behavioral responses (*7*).

Neem extracts and pure azadirachtin are used to control aphids, thrips, fungus gnats, caterpillars, beetles, mushroom flies, mealybugs, leafminers, and gypsy moths, both by contact and ingestion. In Italy, AZA-A is a registered product for strawberry cultivation with a maximum residue level (MRL) of 0.5 mg/kg and a preharvest interval of 3 days.

Field use of these extracts has not always proved highly efficacy against target species, and their low persistence may

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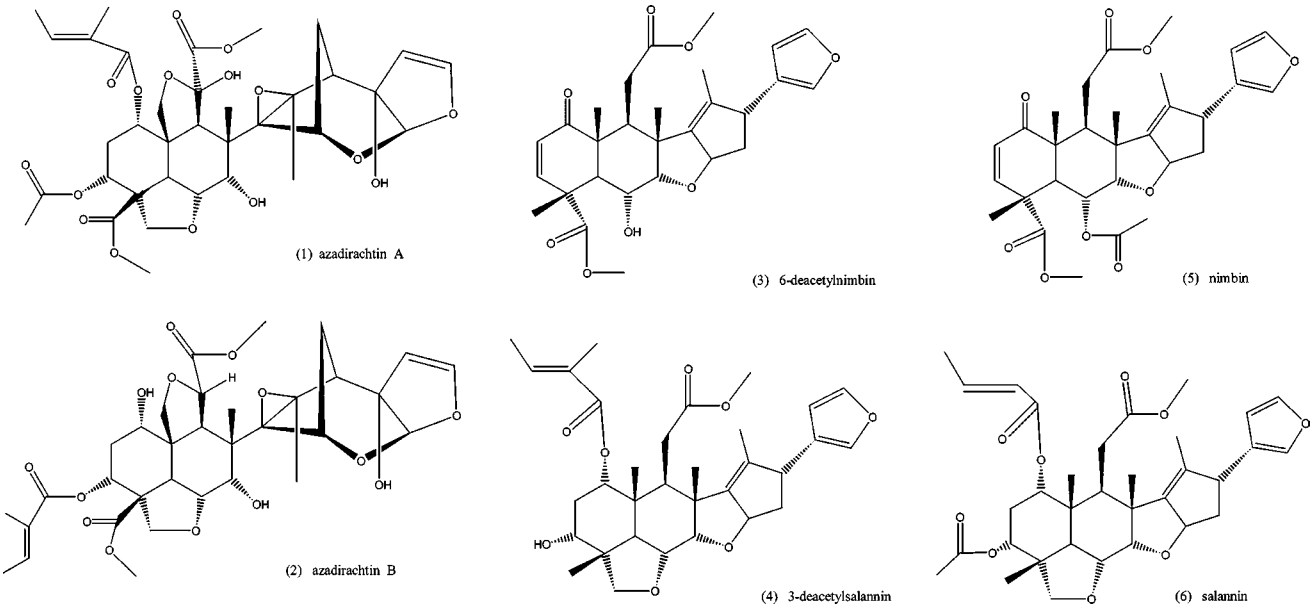

Figure 1. Chemical structures of the main limonoids extracted from the neem seeds.

be mainly ascribable to the low photostability of the formulations commercially available. In a previous study on the fate of azadirachtin on olives, we reported that the commercial formulations showed a lower stability than the active ingredient (a.i.), suggesting that additives in the formulation may accelerate azadirachtin photodegradation (*8*).

The aim of this work was to evaluate residue levels of azadirachtoids on strawberry after field treatment with two different formulations. We have therefore compared photodegradation on a thin layer of the pure limonoids extracted and purified from neem seeds in a model system.

MATERIALS AND METHODS

Chemicals. Acetonitrile (ACN), methanol, and ethanol were of high-performance liquid chromatography (HPLC) grade (Baker, Milan, Italy); hexane, ethyl acetate, and dichloromethane were of gas chromatography grade and purchased from Baker (Milan, Italy); sodium chloride, trifluoroacetic acid 99% (Sigma Aldrich, Steinheim, Germany), propylene glycol, vanillin, and 98% sulfuric acid were from Carlo Erba (Milan, Italy); and water was distilled and filtered through a Milli-Q apparatus (Millipore, Milan, Italy) before use.

The analytical standard of azadirachtin (95% purity) was purchased from Sigma Aldrich, and OIKOS 25 Plus (AZA-A + AZA-B, 25 g/L) and five different neem seed extracts were kindly provided by SIPCAM (Milan, Italy). From the methanolic extract of neem seeds were isolated AZA-A, AZA-B, deacetylnimbin, deacetylsalannin, nimbin, and salannin. Purity and control of the a.i. were carried out by liquid chromatography/mass spectrometry (LC/MS) and NMR. Silica gel 60, i.d. 0.04−0.063 mm, and thin-layer chromatography (TLC) aluminum sheets, 20 cm × 20 cm silica gel 60 F254, were purchased from Merck (Milan, Italy).

Field Trials. Field trails were carried out in a plastic matted row strawberry culture (*Fragaria ananassa* duch cv. Tudla) grown in a 2000 square meters tunnel system. Strawberries were planted in twin rows (three per tunnel) at intervals of 30 cm × 30 cm; each twin row was separated by a 1.0 m alley, and plants were irrigated using a drip system. The field was located near Oristano, Italy.

The experiment was set up in a randomized block design with five replicates of 18 plants per treatment. Treatments were carried out on May 2006 and consisted of: (A) the commercial formulation OIKOS 25 PLUS (SIPCAM) containing AZA-A + AZA-B at 2.5% applied at 1× and 5× the dose recommended by the manufacturer (0.025 g a.i./ha); (B) a laboratory-prepared formulation from the neem seeds extract powder at 1.1% of AZA-A + AZA-B, further applied on field at 0.025 and 0.125 g a.i./ha; and (C) control. Plants were wetted to the drip point using an hand sprayer.

Ripened strawberries (1 kg per block) were harvested from the field for the residue analysis of AZA-A and AZA-B, deacetylnimbin, deacetylsalannin, nimbin, and salannin at levels at time 0 (after treatment to dry plant), 1, 3, 5, and 8 days. Because of the low stability of limonoids when exposed to UV light, all samples were collected in dark plastic bags and analyzed immediately after harvest. During the experiment, the maximum and minimum average temperatures of the ambient air were 31.1 and 12.8 °C, respectively.

Sunlight Photodegradation Experiments. This trial was carried out in June 2006 exposing pure azadirachtoids extracted from the methanolic extract of neem seeds. A portion of the pure a.i. was dissolved in ethyl acetate and poured into Petri quartz dishes of 5 cm diameter to have thin films (0.10 μg/cm^2). To have a uniform film of the a.i. on the dish surface, the solvent was evaporated at room temperature keeping the dishes in the dark at room temperature. The dishes were then exposed to direct sunlight in Cagliari (Italy) at 39°14′ latitude north and 3°20′ longitude west from the Rome Monte Mario meridian and removed at prefixed intervals. Control samples were stored in the dark at room temperature. A control kept in the dark for 20 h gave >95% recovery of the parent compound and no indication of degradation products. After sunlight exposition, the residues of a.i. in the dishes were taken up with 2 mL of ACN and 1 mL of this organic solvent was dried under a gentle nitrogen stream and redissolved with 400 μL of the mobile phase and analyzed by HPLC. Every trial was conducted in four replicates.

Standard Solutions. A stock standard solution of 500 mg/L of AZA-A was prepared in methanol diluting to volume (1 mL) the commercially available standard. A stock standard solution of AZA-B, deacetylnimbin, deacetylsalannin, nimbin, and salannin (1000 mg/L) for all compounds was prepared in methanol by weighing approximately 0.01 g of the pure analyte into a 10 mL volumetric flask and diluting to volume. An intermediary mixed standard solution was prepared daily by diluting with the mobile phase (ACN−water; 35:65, v/v) except for the HPLC/MS analysis (ACN−aqueous 0.1% trifluoroacetic acid; 35:65, v/v). All standard solutions were stored in the dark at −20 °C until usage.

Instrumentation and Sample Analysis. *HPLC/DAD Analysis.* An Agilent Technologies (Waldbronn, Germany) model 1100 high-performance liquid chromatograph was used, fitted with a diode array detector (DAD). An analytical column Waters Spherisorb ODSB (250 mm × 4.6 mm, 5 μm particle size) (Milford, MA) was employed.

For HPLC analysis, an aliquot (100 μL) was injected into the column and eluted at room temperature. For the analytical separation, the

Table 1. LC/MS (ESI^+) Characteristics of AZA-A and Their Related Limonoids

compound	formula	log P^a	HPLC t_R (min)	LC/MS t_R (min)	mw	LC/MS (ESI) *m/z* (% relative abundance)
AZA-A	$C_{35}H_{44}O_{16}$	–0.52	8.19	9.60	720	743 $[M + Na]^+$ 100; 703 $[M + H - H_2O]^+$ 96; 685 $[M + H - 2H_2O]^+$ 80; 585 $[M - 2H_2O - TFA]^+$ 66; 567$[M - 3H_2O - TFA]^+$ 62; 759 $[M + K]^+$ 10
AZA-B	$C_{33}H_{42}O_{14}$	–1.06	8.46	9.99	662	645 $[M + H - H_2O]^+$ 100; 685 $[M + Na]^+$ 85; 726 $[M + Na + ACN]^+$ 52; 709 [unknown] 45; 627 $[M + H - 2H_2O]^+$ 43; 701 $[M + K]^+$ 15
deacetylnimbin	$C_{28}H_{34}O_8$	1.66	16.37	15.40	498	499 $[M + H]^+$ 100; 467[unknown] 22; 562 $[M + Na + ACN]^+$ 9
deacetylsalannin	$C_{32}H_{42}O_8$	1.89	17.29	15.67	554	555 $[M + H]^+$ 100; 601 [unknown] 81; 618 $[M + Na + ACN]^+$ 12; 573 $[M + H + H_2O]^+$ 8
nimbin	$C_{30}H_{36}O_9$	2.59	19.66	18.70	540	541 $[M + H]^+$100; 604 [unknown] 30; 509 [unknown] 25; 481 [unknown] 10
salannin	$C_{34}H_{44}O_9$	2.82	22.42	20.35	596	597 $[M + H]^+$ 100; 619 $[M + Na]^+$ 15; 660 $[M + Na + ACN]^+$ 15

[a] Log *P* values were calculated with CS ChemDraw Pro Cambridge Software Corp. (Cambridge, MA).

gradient profile of the mobile phase, at the constant flow of 1 mL/min, was as follows: initial (35:65, v/v) ACN−water reaching (50:50 v/v) in 15 min and hold to 40 min. Before the next injection, the HPLC system must be stabilized for 10 min with ACN−water (35:65 v/v). Detection was carried out scanning wavelengths between 200 and 400 nm, and quantification involved peak area comparisons with analytical standards and absorbance measurements at 215 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. The isocratic elution was with ACN−aqueous 0.1% trifluoroacetic acid 99% (60:40, v/v) for 30 min. The column used was a Waters XTerra MS RP18 (250 mm × 2.1 mm, 5 μm particle size). The injection volume was 20 μL, and the flow rate was 0.4 mL/min. The electrospray ionization (ESI)-MS interface was operated in the positive ion mode: ESI source probe, 250 °C; CDL, 250 °C; block at 200 °C; flow gas (N_2) at 2.5 mL/min; probe voltage, 3 kV; scan, 250−850 amu; and SIM mode acquiring 703, 645, 499, 541, 555, and 597 amu for AZA-A and AZA-B, deacetylnimbin, nimbin, deacetylsalannin, and salannin respectively.

NMR Experiments. NMR liquid state ^1H and ^{13}C NMR spectra (300 MHz) were recorded with a VARIAN VXR300 spectrometer, console SUN 3/60, operative system SOLARIS/UNIX, equipped with a 10 mm $^1H-^{13}C$ probe. Samples were dissolved in deuterated chloroform in 5 mm wilmad tubes containing 0.05% of the internal standard tetramethylsilane.

Extraction Procedure from Fruits and Vegetables. A portion (5 g) of well-homogenized chopped strawberries was weighed in a 40 mL screw-capped glass tube, and 4 g of sodium chloride and 20 mL of ACN were added. The tube was agitated for 15 min in a rotary shaker at 9 rpm (FALC Instrumentals, Bergamo, Italy) at room temperature, and 1 mL of the mixture was evaporated to dryness under a gentle nitrogen stream. The residue was dissolved with 200 μL of the mobile phase (65:35, water/ACN v/v) and submitted for HPLC-DAD and liquid chromatography/mass spectrometry (LC/MS) analysis. The amount of sample in the final extract was 1.25 g/mL.

Recovery Assays. A 50 μL aliquot of pesticide solution at the desired standard concentration was added to each 5 g sample of untreated strawberries. The fortification levels used were 0.05, 0.10, 0.50, 1.0, and 5.0 mg/kg. The samples were allowed to settle for 30 min prior to extraction. They were later processed according to the above extraction procedure. Four replicates for each level were analyzed by HPLC-DAD and LC/MS.

Vacuum Liquid Chromatography (VLC) Fractioning. The dried and pulverized neem seed methanolic extract (10 g) was subjected to fractionation by VLC; the 30 cm glass column (i.d. 5 cm) packed with silica gel (82.5 g) was eluted successively with solvent mixtures of increasing polarity (hexane, dichloromethane, ethyl acetate, and methanol) starting from 100% hexane to 100% methanol. Fractions obtained in this way were further fractionated and purified by open column chromatography (CC) on silica gel, yielding six limonoids, namely, AZA-A, AZA-B, deacetylnimbin, deacetylsalannin, nimbin, and salannin. A chromogenic solution consisting of (a) 95:5 ethanol−sulfuric acid (v/v) and (b) 99:1 ethanol−vanillin (v/w) was used to reveal compounds of interest on TLC.

Isolation of Azadirachtoids. *Isolation of AZA-A.* The fraction containing AZA-A was purified with a 60 cm glass column (i.d. 4 cm) packed with silica gel (90 g). The column was then eluted with a mixture of ethyl acetate−hexane (80:20 v/v). Fractions containing impure AZA-A were further purified using a 50 cm glass column (i.d. 2 cm) packed with silica gel (30 g). The column was then eluted with a mixture of ethyl acetate−hexane (70:30 v/v) yielding 106 mg of AZA-A.

Isolation of AZA-B. The fraction containing AZA-B was purified with a 60 cm glass column (i.d. 4 cm) packed with silica gel (90 g). The column was then eluted with a mixture of ethyl acetate−hexane (60:40 v/v) yielding 132 mg of AZA-B.

Isolation of Deacetylnimbin. The fraction containing deacetylnimbin was purified with a 60 cm glass column (i.d. 4 cm) packed with silica gel (90 g). The column was then eluted with a mixture of ethyl acetate−hexane (40:60 v/v) yielding 26 mg of deacetylnimbin.

Isolation of Deacetylsalannin. The fraction containing deacetylsalannin was purified with a 60 cm glass column (i.d. 4 cm) packed with silica gel (90 g). The column was then eluted with a mixture of ethyl acetate−hexane (60:40 v/v). Fractions containing impure deacetylsalannin were further purified using a 50 cm glass column (i.d. 2 cm) packed with silica gel (30 g). The column was then eluted with a mixture of ethyl acetate−hexane (60:40 v/v) yielding 14 mg of deacetylsalannin.

Isolation of Nimbin. The fraction containing nimbin was purified with a 60 cm glass column (i.d. 4 cm) packed with silica gel (80 g). The column was then eluted with a mixture of ether petroleum−diethyl ether (50:50 v/v) yielding 42 mg of nimbin.

Isolation of Salannin. The fraction containing salannin was purified with a 60 cm glass column (i.d. 4 cm) packed with silica gel (90 g). The column was then eluted with a mixture of ethyl acetate−hexane (40:60 v/v) yielding 11 mg of salannin.

RESULTS AND DISCUSSION

HPLC-DAD and LC/MS Analysis. HPLC and LC/MS were the critical methods for compound identification, matching t_R values and ESI fragmentation patterns with the limonoid standards isolated and purified from the methanolic seed extract. HPLC and LC/MS fragmentation characteristics of azadirachtoids are reported in **Table 1**.

From the HPLC-DAD analysis at 215 nm, which was chosen because this value agrees with the λ_{max} (between 209 and 217 nm) of all analytes, the seed extracts analyzed (C−G) (**Table 2**) had on average a higher content of salannin (19.01%) and nimbin (10.45%) while the percent contents of deacetylsalannin

Table 2. Percent Content of Main Azadirachtoids in the Methanolic Extracts of the Neem Seeds and in the Formulations A and B Used for Field Treatments

	formulation						
compound	A	B	C	D	E	F	G
AZA-A	1.50	0.70	7.76	11.33	12.22	9.87	13.20
AZA-B	0.54	0.44	4.15	3.96	3.58	3.03	2.96
deacetylnimbin	0.47	0.38	4.03	2.68	3.65	2.54	2.75
deacetylsalannin	0.52	0.47	4.48	2.78	4.25	3.13	3.42
nimbin	1.44	0.92	12.17	10.08	11.11	9.12	9.75
salannin	3.07	1.06	11.71	22.19	24.42	17.71	19.10
sum of azadirachtoids	7.54	3.97	44.30	53.02	59.23	42.40	51.18

and deacetylnimbin were 3.61 and 3.13, respectively. The values for AZA-A and AZA-B in the same extracts were 10.88 and 3.54%, respectively, whereas percent contents of AZA-A, AZA-B, deacetylnimbin, deacetylsalannin, nimbin, and salannin in the commercial formulation A were 1.50, 0.54, 0.47, 0.52, 1.44, and 3.07, respectively. A HPLC-DAD chromatogram of the methanolic neem seeds extract used to prepare the formulation B for field treatment and an LC/MS chromatogram of the six limonoids of interest at 1 mg/L are reported in **Figure 2**.

LC-ESI-MS in the positive ion mode allowed the quantification and the separation of all azadirachtoids in the strawberry extract because of an interfering peak in the analytical region of interest. The use of the mass detector working in the single ion monitoring (SIM) mode together with DAD detector set at 215 nm wavelength revealed to be successful. Limits of quantitation (*9*) for AZA-A and AZA-B, deacetylnimbin, deacetylsalannin, and salannin in the SIM mode were 0.01 mg/kg except for nimbin (0.5 mg/kg). Recoveries for all compounds tested were >78% in the concentration range of 0.05−5 mg/kg. Good linearity was achieved for all of the compounds tested in the concentration range from 0.01 to 5 mg/kg with correlation coefficients between 0.9991 and 0.9999. For compound quantitation were chosen the most abundant adducts in the LC/MS spectra. **Figure 3** shows LC/MS spectra of azadirachtin and their analogues.

Table 3. Half-Life Time $t_{1/2}$ (h) of Azadirachtoids after Sunlight Exposure as Thin Films

compound	$t_{1/2}$ (h)	r
AZA-A	11.34	0.989
AZA-B	5.52	0.997
deacetylnimbin	0.11	0.993
deacetylsalannin	0.21	0.983
nimbin	0.30	0.989
salannin	0.09	0.998

^{1}H and ^{13}C NMR spectra and chemical shifts for all compounds isolated from the seed extract were comparable with those reported by Johnson et al. (*10*) and Barrek et al. (*11*).

Sunlight Photodegradation Experiments in a Model System. Dureja and Johnson (*12*) reported that AZA-A when exposed to UV light (254 nm), as a solid thin film on a glass surface, undergoes isomerization from a *cis*-(*Z*) to a *trans*-(*E*) configuration with a half-life time of 48 min. In our experiment, when AZA-A and AZA-B were exposed as a thin film under sunlight, the degradation half-lives were of 11.3 (r = 0.989) and 5.5 h (r = 0.997), respectively. In these experimental conditions, half-life times for salannin, nimbin, deacetylsalannin, and deacetylnimbin were in the order of minutes: 5.7 (r = 0.998), 18.3 (r = 0.989), 12.7 (r = 0.983), and 6.5 min (r = 0.993), respectively (**Table 3**).

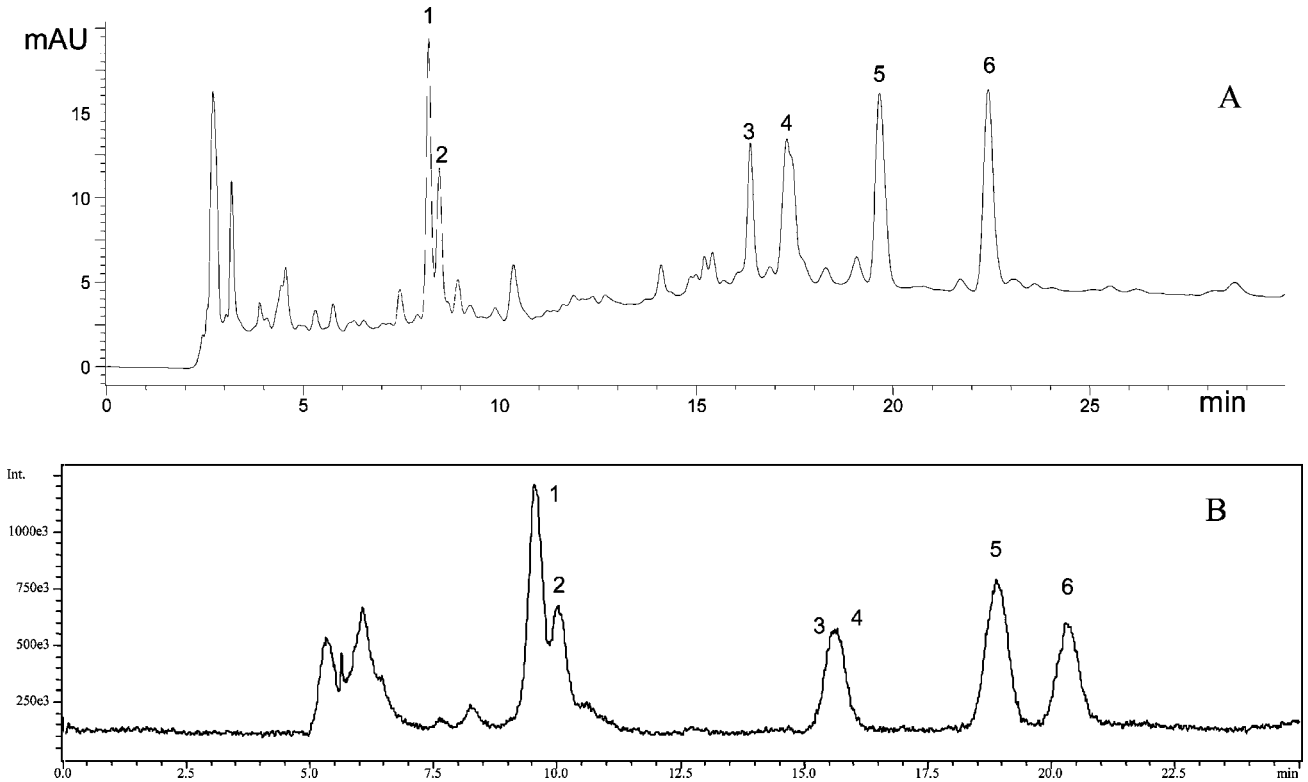

Figure 2. (**A**) HPLC-DAD chromatogram at 215 nm of the methanolic extract of neem seed. Azadirachtoids were chromatographically separated with the following elution order: 1, AZA-A; 2, AZA-B; 3, deacetylnimbin; 4, deacetylsalannin; 5, nimbin; and 6, salannin at 1 mg/L. (**B**) LC-ESI-MS chromatogram of the six analytical standards.

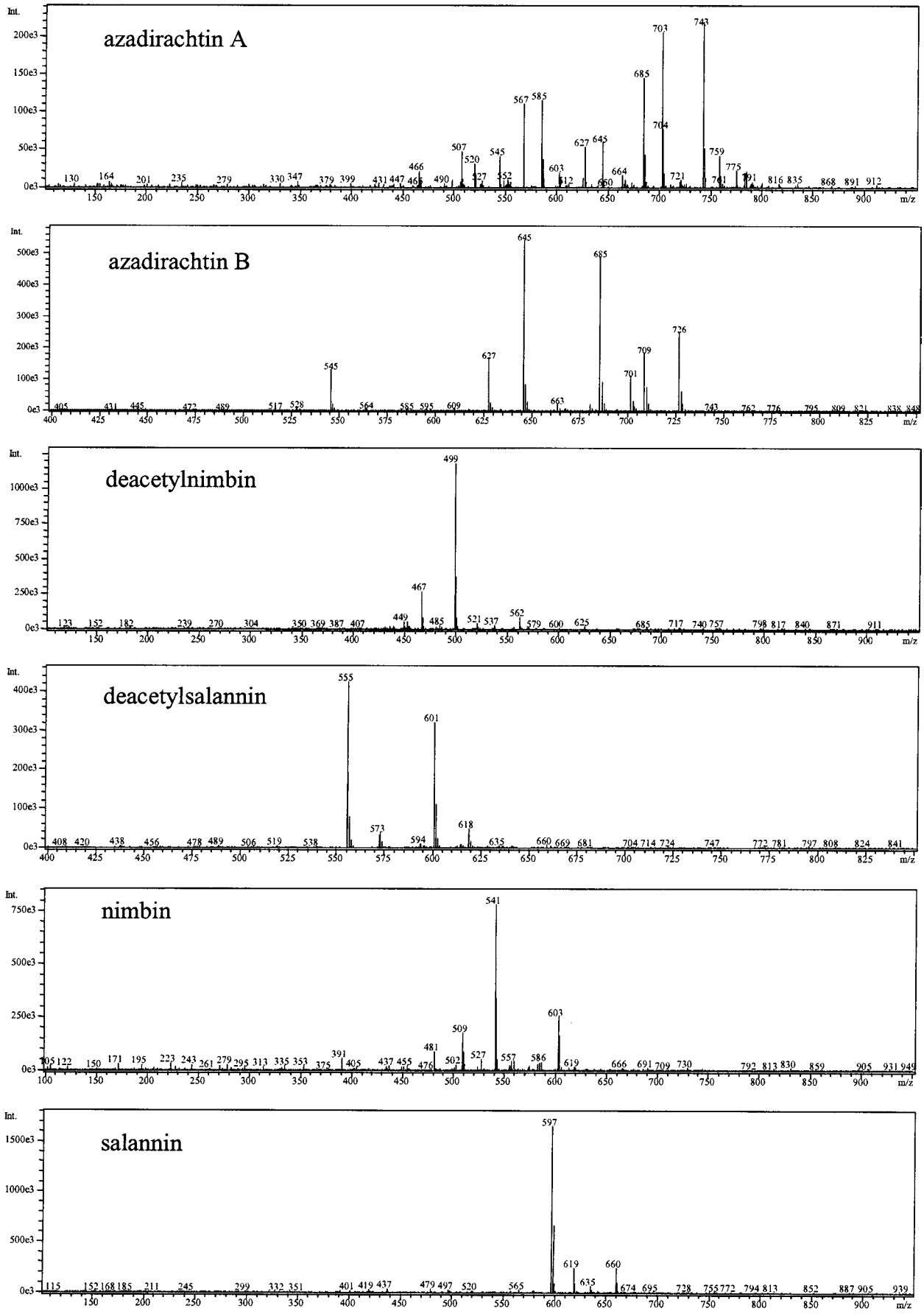

Figure 3. LC/MS spectra of azadirachtins and their analogues: AZA-A, 1; AZA-B, 2; deacetylnimbin, 3; deacetylsalannin, 4; nimbin, 5; and salannin, 6.

Table 4. Residues (mg/kg ± SD, $n = 5$) and Half-Life Times of Azadirachtoids on Strawberries after Field Treatments at 0.125 g a.i./ha for Two Different Formulations[a]

time (days)	AZA-A	AZA-B	deacetylsalannin	deacetylnimbin	salannin	sum of azadirachtoids
			formulation A			
0	0.50 ± 0.08	0.09 ± 0.03	0.25 ± 0.08	0.08 ± 0.02	0.48 ± 0.09	1.40
1	0.18 ± 0.04	0.04 ± 0.02	0.09 ± 0.02	0.04 ± 0.01	0.07 ± 0.03	0.42
3	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.00	<0.01	<0.01	0.06
5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
$t_{1/2}$ (days)	0.73 ($r = 0.999$)	0.97 ($r = 0.999$)	0.84 ($r = 0.995$)	NC	NC	
			formulation B			
0	0.28 ± 0.05	0.07 ± 0.02	0.09 ± 0.05	0.06 ± 0.01	0.13 ± 0.03	0.63
1	0.20 ± 0.02	0.07 ± 0.02	0.09 ± 0.02	0.03 ± 0.01	<0.01	0.39
3	0.22 ± 0.04	<0.01	0.02 ± 0.01	<0.01	<0.01	0.30
5	0.06 ± 0.01	<0.01	<0.01	<0.01	<0.01	0.06
8	0.02 ± 0.01	<0.01	<0.01	<0.01	<0.01	0.02
$t_{1/2}$ (days)	2.05 ($r = 0.956$)	NC	1.28 ($r = 0.955$)	NC	NC	

[a] NC, not calculated.

Field Trials. In Italy, the fixed MRL for AZA-A is 0.5 mg/kg in all crops with a safety interval of 3 days. Residues of azadirachtoids on strawberry after field treatment are given in **Table 4**.

With the 1× treatment at the dose recommended by the manufacturer, it was impossible to quantitate limonoids because their concentrations were below the limit of quantitation. Further information on the behavior of limonoids on field was assessed with five times (5×) the recommended dose. After treatment with the commercial formulation (A) in the 5× experiment, the AZA-A content was 0.50 mg/kg, which was close to the MRL, and the sum of detectable azadirachtoids was 1.40 mg/kg. The total content of azadirachtoids in the 5× experiment using the formulation (B) was 0.63 mg/kg, and the AZA-A content was 0.28 largely under the fixed MRL. Even though the applied dose was the same for the two formulations, the AZA-A level on strawberry with the formulation B was approximately 50% as compared with formulation A, and this is probably due to the different adjuvants used. Surfactant properties of the formulation B can be ameliorated to improve spray droplet deposition and adhesion to leaf and fruit surfaces.

Residues and half-lives of nimbin were not measured because of the high limit of quantitation (LOQ 0.5 mg/kg) in the strawberry extract. Because the size of the fruits did not change during the experiment, the decrease of the pesticides due to a dilution effect can be ruled out.

Persistence of active ingredients in the commercial formulation was compared with the same active ingredients in a laboratory formulation using adjuvants allowed in organic farming. The rate of disappearance for the two formulations appeared to be different in the experiment conducted with 5× treatment. In the formulation A, AZA-A and AZA-B showed half-lives of 0.73 and 0.97 days, respectively, while deacetylsalannin showed a half-life of 0.84 days. While in the photodegradation study of the formulation B, AZA-A showed a persistence 2.8 times higher if compared with formulate A. In the formulation B, AZA-A was more photostable than the commercial formulation. The sum of the different azadirachtoids during the experiment is mainly due to the more stable limonoids and may be due to different additives (excipients) present in the formulations.

Moreover, half-life times of azadirachtoids in the laboratory photodegradation experiment were lower than those of field samples, suggesting a greater photostability of the formulation tested.

ABBREVIATIONS USED

DAD, diode array detector; LC/MS, liquid chromatography/mass spectrometry; ESI, electrospray ionization; ACN, acetonitrile; TLC, thin-layer chromatography; VLC, vacuum liquid chromatography; AZA-A, azadirachtin A; AZA-B, azadirachtin B; a.i., active ingredient.

ACKNOWLEDGMENT

Helpful suggestions and help were provided by Dr. F. Cottiglia, Dr. G. Uccheddu, Dr. A. Barra, and A. Demuro.

NOTE ADDED AFTER ASAP PUBLICATION

The original posting of November 22, 2006, contained an incomplete version of Figure 3. This has been corrected with the posting of November 28, 2006.

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Received for review August 28, 2006. Revised manuscript received October 9, 2006. Accepted October 10, 2006.

JF062461V