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PESTICIDE RESIDUES IN IMMATURE SOYBEANS OF ARGENTINA CROPLANDS

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SUMMARY

Residue levels of endosulfan, endosulfan sulphate, glyphosate, and aminomethylphosphonate (AMPA) in green turgescent immature seeds of glyphosate-tolerant and non-tolerant soy beans were studied in Argentina. To establish the incidence of pesticides` spraying, one control and two plots that varied in glyphosate and endosulfan treatments in two different fields, located in Entre Ríos Province (Argentina), were studied. Endosulfan, glyphosate and AMPA are present in immature soybeans and analyses revealed that the concentration of endosulfan residues were similar between soy varieties, but statistical differences were observed between the two treatments.

KEYWORDS:
Immature soybean, pesticide residues, endosulfan, glyphosate.

INTRODUCTION

Despite most soybeans are harvested at the mature stage and further processed into a variety of products for human or animal uses, in certain areas of the world, such as oriental countries, considerable quantities are harvested at the immature stage for direct human consumption [1]. The immature soybeans refer to those harvested at 80 % of maturity and their seeds have low dried matter [1, 2]. This maturity level is equivalent to R_6 stage [3].

The immature soybean is often used directly as human food in Asia, because of the high level of nutrients, carbohydrates, vitamins, and isoflavones. Actually, soy is a global staple food and more than 110 million tons of beans are produced, mainly in the United States, Brazil, Argentina, and China [4].

In soy production, a pre-planted application with glyphosate ((N-(phosphonomethyl)glycine) [5] is recommended, followed by one or two applications of this herbicide on pre-flowered glyphosate-tolerant (GT) soybeans [6], but anyone on conventional (CN) soybeans [7]. Moreover, an organochlorine pesticide (endosulfan) is also applied twice or even more times in the seeding stage to control sucking, chewing and boring insects and mites [8, 9].

Recent research reports and articles on the agronomic and environmental benefits and risks of GT-soybean have been published [10]. In this context, soybean has been recognised to have a very significant contribution in nutrition and human health suggesting that consumption of soybean products is associated with lower blood cholesterol, gives protection against cardiovascular disease, and reduces risk of certain cancers and osteoporosis [11]. On the other hand, preliminary studies observed that pesticides` residues usually used in soy cultivation are present in milk, dairy products, and wild fauna [12-15] and have negative effects on human and wildlife health [16].

Therefore, we analyzed pesticide residues present in immature soybeans (IS) and compared GT-soybean vs. CN-soybean. In addition, a new technique was used to evaluate endosulfan, endosulfan sulphate, glyphosate, and alpha-amino-3-hydroxy-5-methylisoxazolepropionate (aminomethylphosphonate, AMPA). The study was conducted to assess whether the use of glyphosate on GT-soybean and endosulfan on GT- and CN-soybean affect the residues` concentration of pesticides in immature seeds. This study is part of a major project, which investigates the persistence of pesticides used in agricultural production and the biomagnification of residues through the highest food chain.

MATERIALS AND METHODS

Spray treatments

The study was conducted at the Entre Ríos province (Argentina) in two experimental fields located at Sauce Pinto (31°52'S; 59°30'W) and Diamante (31°58'S; 60°32'W) through the soy seasons from November 2000 to March 2001. During the study the average minimum and maximum temperatures ranged between 17.9 °C - 32.3 °C in 2000, and 30.4 °C - 19.2 °C in 2001. GT-soybean in Diamante (20 ha) and CN-soybean in Sauce Pinto (30 ha), both seeding in December. Glyphosate (Roundup®, Monsanto, Argentina) was applied seven days after seeding (2500 g ha^{-1}) on GT-soybean, and no spraying on CN-soybean. In each experimental field were considered three plots, Plot 1: endosulfan free (control plot), Plot 2: endosulfan applied once on soy 92 days after seeding, and Plot 3: two endosulfan sprayings on 60^{th} and 92^{nd} day after seeding (R_6 stage). In all the cases the spraying solution was applied at 1000 g ha^{-1}. The endosulfan (Agarcros, Argentina) was prepared by appropriate dilution with water of the commercial herbicide Piastra®, 350 g $litre^{-1}$.

Sampling procedure

For the study, both soybean crops were harvested at random 100 days after seeding from the field at the R_6 stage of development. Replicate bean samples were collected in each plot and analyzed separately to determine the pesticide residues (α+β-endosulfan, endosulfan sulphate, glyphosate, and AMPA). Immediately after picking each sample, the beans were packed in polyethylene bags, transported to the laboratory to be worked up or frozen at – 18 °C within 90 min.

Analytical procedure

Twenty grams of samples of immature beans were extracted as follows: weighed sample was macerated in a homogeneizer at 17,000 rpm. with ultra-pure water (100 ml) to extract glyphosate and AMPA, transferred into a centrifuge tube with 10 ml ultra-pure tap water and then 50 ml ethyl acetate were added. After been shaken for 10 min, the samples were centrifuged at 7,000 rpm for 10 min at room temperature.

From the clarified supernatant, an aliquot from the organic layer (40-45 ml) was taken and filtered through anhydrous sodium sulfate, concentrated to 1-2 ml by rotary evaporator (Büchi, Switzerland or similar) at 40 °C and analyzed by gas chromatography (GC) for endosulfan and endosulfan sulphate. Aliquots of water layer (5ml), filtered by using 0,45 µm pore size, was analyzed for glyphosate and AMPA by high performance liquid chromatography (HPLC).

GC and GC/MS analysis: Varian; model 3400, with electron-capture detector (ECD) Ni^{63}, DB-5 capillary column 30 m x 0.25 mm ID (J&W Scientific CA, USA), injector 220 °C, oven 250 °C and ECD detector 320 °C. Mass spectra were made with a Varian 2000 chromatograph-mass selective detector (MSD) combination, equipped with an HP-5 capillary column (30 m x 0.25 mm ID) to confirm GC results (operational conditions: oven 210°C, injector 230 °C, ion trap mass spectrometer operating in electron ionization (EI) mode and using MS/MS option.

HPLC analysis: Waters 600 with IC-Pack ion-exclusion 50 Å - 7µm (7.8 x 150 mm) column; mobile phase phosphoric acid 0.05% at 55 °C, fluorescence detector at excitation 339 and emission 345 nm, and Millenium 2000 data processor software.

Chemical pesticide standards, α and β-endosulfan, endosulfan sulphate (99.0% purity) glyphosate and AMPA (99.2% purity, Applied Science, USA) were used.

Statistical analysis

Analyses of variance (ANOVA) were carried out on the data obtained experimentally (residue concentrations) to evaluate the influence of soy transgenic and non-trangenic variety (variety factor) and number of endosulfan spraying on soy samples (treatment factor).

RESULTS AND DISCUSSION

In order to obtain the calibration linearity, standard solutions at 0.2, 0.5, 1.0, 10.0 and 50.0 µg $litre^{-1}$ were used for endosulfan isomers (α and β) and endosulfan sulphate, with dieldrin as internal standard. Nevertheless, 0.5, 1.0, 2.0 and 8.0 µg $litre^{-1}$ of glyphosate and 0.45, 1.0, 2.0, and 3,6 µg $litre^{-1}$ of AMPA were used. Three replicates of each concentration were analyzed and detection limits calculated. The data revealed no statistical differences in the intercept and slopes obtained for each one of the five analytes studied ($p > 0.05$).

Recovery studies were performed using clean soybean samples (20 g) fortified with two concentrations of endosulfan and endosulfan sulphate, (20 and 40 µg kg^{-1}) glyphosate and AMPA (3.2 and 6.0 mg kg^{-1}).

A rapid and efficient method for simultaneous extraction and quantification of pesticide residues has been applied for the analysis of immature soy seeds (IS), in a routine pesticide residue laboratory [18, 23]. Good recoveries were obtained for endosulfan isomers, endosulfan sulphate, glyphosate and AMPA (92.3%; 96.1%; 92.1%; 95.1%, respectively) with a relative standard deviation (RSD) lower than 11.7%. The detection limits were found to be 5 10^{-4} mg/kg for endosulfán group and 5 10^{-3} mg/kg for the other herbicides and metabolites.

Residues found in the IS samples after one and two endosulfan applications on GT- and CN-soybeans are shown in Table 1. The residues of endosulfan applied as spray on the plants, were found in the immature beans, but endosulfan sulphate was no detected (n.d.).

The effect of “variety” was calculated only for endosulfan residues. This effect was not significant to endosulfan residues, because analysis of variance showed no significant difference between GT- and CN-soybeans (p = 0.8832). On the contrary, “treatment” significantly affected endosulfan concentrations ($p < 0.01$), but the effect was not significant for glyphosate residues (Table 2), as expected.

TABLE 1
Residues (mg/kg) in immature GT-soybean and CN-soybean. SD = standard deviation; n.d. = not detected; Plot 1: endosulfan-free (control plot); Plot 2: 92 days after seeding; Plot 3: 60 and 92 days after seeding (R_6 stage); a, b indicate significant differences at $p < 0.05$.

		Residues							
Soy	Endosulfan spraying	Endosulfan		Endosulfan sulphate		Glyphosate		AMPA	
		mg/kg	SD	Mg/kg	SD	mg/kg	SD	mg/kg	SD
GT	Plot 2	0.18 a	0.03	n.d.	-	0.31a	0.04	0.33	0.03
GT	Plot 3	0.24 b	0.04	n.d.	-	0.30a	0.05	0.32a	0.04
GT	Plot 1	n.d.	-	n.d.	-	n.d.	-	n.d.	-
CN	Plot 2	0.19a	0.05	n.d.	-	n.d.	-	n.d.	-
CN	Plot 3	0.23ab	0.04	n.d.	-	n.d.	-	n.d.	-
CN	Plot 1	n.d.	-	n.d.	-	n.d.	-	n.d.	-

TABLE 2
Effect of number pesticide treatment on endosulfan and glyphosate residues. Effect of soy variety (transgenic or conventional) on endosulfan residues in immature soybeans are also shown; ($p < 0.05$).

	Endosulfan		Glyphosate	
	F	"p"	F	"p"
Treatment	7.96	0.0079	8.09	0.0120
Variety	0.02	0.8832	-	-

- not calculated

Many studies were carried out to establish the curve of decline of the insecticide in vegetables for human consumption [17,18] Thus, Soto [19] and Lu [20] suggested that endosulfan has estrogenic effects on human estrogen-sensitive cells. We found that the GT-soybeans accumulated glyphosate in their immature seeds (0,3 - 0,31 mg/kg) and AMPA metabolites were also generated (0,32-0,33 mg/kg), whose toxicity is discussed elsewhere [21]. In CN-soybeans herbicide residues and corresponding metabolites were absent. The glyphosate and AMPA residues found in GT-soybean were quite similar, and higher than the limits permitted by European legislation (0.2 mg/kg) in the Codex Alimentarius [22], while the Federal Register of Argentina [23] contains no regulations.

The $\alpha+\beta$ endosulfan residues (0.18 to 0.24 mg/kg) were found to be below those recommended by European legislation for dry soybean [22], but there is no regulation with regard to IS. Moreover, the endosulfan sulfate metabolite, which is known as degradation product in mature vegetables, was not detected in this study.

The market outlook concerning IS shows increasing tendencies to be incorporated in Asian and American foods. In this context, an effort should be made to regulate the production, processing and marketing of IS. Similarly, it is necessary to study the possible replacement of endosulfan by a pyrethroid in order to reduce contamination and cost in the production of IS. In addition, the use of glyphosate should be reduced and the weeds extraction may be carried out manually in small fields and in countries with low-cost workmanship standards, in order to reduce the toxic concentrations of biocides.

Argentinean farmers seek to maximize production, on the one hand, and lower production costs, on the other, because the growing conditions vary considerably from one area to another. In deed, from farm to farm, production cost and corresponding savings can vary substantially in this context. Therefore, the disjunctive use of GT-soybean or CN-soybean depended on its world-wide differing acceptance, taking into consideration also the current debate on transgenic herbicide-resistant soybean varieties and their ban, particularly in Europe.

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