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Pesticide residues in Junca Onion (*Allium fistulosum*) cultivated in Risaralda, Colombia

Resíduos de pesticidas na Cebola Junca (Allium fistulosum) cultivada em Risaralda, Colômbia

Juan Pablo Arrubla Vélez[1*]; Nathaly Villa Pulgarín[2]; Eddi Arsail Grisales Betancur[3]; Jose Manuel Grisales Bedoya[3]; Melissa Andrea Gómez Benitez[4]; Gloria Edith Guerrero Álvarez[5]; Diego Paredes Cuervo[6]

Highlights:
Simultaneous detection of 39 organophosphates (OPPs) and organochlorines (OCPs) pesticides was developed.
The method was succesfully applied to determine pesticides in real samples of junca onion.
The LOD of the method ranged from 0.11-7.15 μg kg^{-1} with acceptable precision and accuracy.
A greater presence of organochlorine pesticides banned or severely restricted by the Rotterdam agreement were detected in samples.

Abstract

By 2050, the world population will reach 9.2 billion, increasing the food demand by twice. Lowering loss due to pests is still challenging, where pesticides play an important role, but its indiscriminate use causes inadequate residual amounts to be present in foods. This study aims to monitor the organochlorine and organophosphorus pesticide residuality in *Allium fistulosum* cultivated in Risaralda, Colombia using gas chromatography - mass spectrometry (GC-MS). This method presented a highly sensitive (LOD: 0.11-7.15 μg kg^{-1}), acceptable precision (RSD: 0.83-1.35%) and recoveries percentages between 46.32% - 118.67%. A greater presence of organochlorine pesticides banned or severely restricted by the Rotterdam agreement, such as 4,4'-DDT, was reported in samples of *Allium fistulosum*, with concentrations up to 221.22 μg kg^{-1}, while endrin with a concentration of 469.23 μg kg^{-1} and its degradation products which exceed the maximum residue limite (MRL) for plant samples, reported by the Codex Alimentarius. According to this MRL, it was found that 73.1% of the samples have residual exceeding the allowed limit of organochlorine pesticides by more than forty times, posing a risk to human health and the ecosystem. Continuous monitoring and strict governmental control are required to reduce the exposure of humans and other living beings.

Key words*: Allium fistulosum.* Chromatography. MR. Organophosphorus pesticide. Organochlorine pesticide.

[1] Prof. Dr., Escola de Tecnologia Química, Grupo de Pesquisa Oleoquímico, Universidad Tecnológica de Pereira, Pereira, Colombia. E-mail: juanpablo77@utp.edu.co
[2] Assistente de Investigação, Grupo de Pesquisa Oleoquímico, Universidad Tecnológica de Pereira, Pereira, Colombia. E-mail: nvilla@utp.edu.co
[3] Química Industrial, Escola de Tecnologia Química, Grupo de Pesquisa Oleoquímico, Universidad Tecnológica de Pereira, Pereira, Colombia. E-mail: eddark01@gmail.com; josegrisales@utp.edu.co
[4] Dr. Assistente de Investigação Faculdade de Ciências Ambientais Grupo de Pesquisa em Água e Saneamento, Universidad Tecnológica de Pereira, Pereira, Colombia. E-mail: meangomez@utp.edu.co
[5] Prof. Dr., Escola de Tecnologia Química, Grupo de Pesquisa Oleoquímico, Universidad Tecnológica de Pereira, Pereira, Colombia. E-mail: gguerrero@utp.edu.co
[6] Prof. Dr., Faculdade de Ciências Ambientais Grupo de Pesquisa em Água e Saneamento, Universidad Tecnológica de Pereira, Pereira, Colombia. E-mail: diparede@utp.edu.co
[*] Author for correspondence

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Resumo

Em 2050 a população mundial chegará a 9.2 bilhões, aumentando a demanda por alimentos em duas vezes. Diminuir as perdas devido a pragas ainda é um desafio, onde os pesticidas desempenham um papel importante, mas seu uso indiscriminado faz com que quantidades residuais inadequadas estejam presentes nos alimentos. Este estudo tem como objetivo monitorar a concentração residual de pesticidas organoclorados e organofosforados em cebola junca (*Allium fistulosum*) cultivada na Colômbia, como ou uso de GC-MS. O método usado tem alta sensibilidade (LOD: 0.11-7.15 μg kg^{-1}), precisão aceitável precision (RSD: 0.83-1.35%) e percentagens de recuperações entre 46.32% - 118.67%. Pesticidas clorados proibidos pela Convenção de Roterdã foram encontrados nas amostras com concentrações acima de 221.22 μg kg^{-1}, enquanto endrin com uma concentração de 469.23 μg kg^{-1} e seus produtos de degradação que excedem o limite máximo de resíduos (LMR) para amostras de plantas. De acordo com o LMR do Codex Alimentarius, constatou-se que 73.1% das amostras têm residual superior ao limite permitido de pesticidas organoclorados em mais de 40 vezes, o que representa um risco para a saúde humana e ecossistema. Monitoramento contínuo e rigoroso controle governamental são necessários para reduzir a exposição de seres humanos e outros seres vivos.

Palavras-chave: Allium fistulosum. Cromatografia. Pesticidas.

Introduction

According to the [FAO] (2009), the world population projects an increase of up to 9.2 billion people in 2050. The continuous increase in population results in an increase in for the demand for food and studies suggest that world crop production should double by 2050. However, at the current rate of growth, this production goal will not be achieved to meet the projected demands of population growth, changes in diet and the increase in the use of biofuels (Ray, Mueller, West, & Foley, 2013). One of the greatest challenges faced is that the agricultural land is limited, and agricultural expansion would have to occur at the expense of clearing forests and depleting the natural habitat of wildlife. Therefore, an increase in sustainable production and the productivity of existing lands is the best option, since the reduction of yield losses caused by plagues is one of the main challenges for agricultural production (Popp, Pető, & Nagy, 2013).

Pesticides are an immediate option for controlling crop pest problems (Gomiero, Paoletti, & Pimentel, 2008). Ideally, a pesticide must be lethal for target pests but harmless for the non-taget species, including human beings. But this is not usually the case and controversy has arisen over the use and abuse of pesticides, which has caused a havoc in the ecosystem and even in the lives of humans (Aktar, Sengupta, & Chowdhury, 2009).

Allium is a genus of onions with 600–920 species (Peterson, Annable, & Rieseberg, 1988). It is a globally harvested crop with an annual harvest of 98,893,680 tonnes (FAOSTAT, 2016). The junca onion (*Allium fistulosum*) has great economic importance in Colombia with a production of 289,975 metric tonnes. This crop is attacked by several pests, and among the most common are Chisas (*Ancognata scarabaelodes* Burmeister), cutters worms (*Agrotis ipsilon* Hufnagel), slugs and snails (*Deroceras reticulatum* Müller, small slugs, *Limax marginatus* Müller, striped slugs, *Milax gagates* Draparnaut, grey slugs, *Helix aspersa*, snails, onion root flies or root neck borers (*Delia* sp), Trips (*Thrips tabaci* and *Frankliniella occidentalis*) and Onion miners (*Liriomyza huidobrensis*), among others (Departamento Administrativo Nacional de Estadística [DANE], 2015). The use of pesticides is a determining factor in the yield of onion crops due to the diversity of pests and diseases that attack it (Gent et al., 2006).

The chemical inputs commonly used as methods for eradication of diseases and pests in crops include organochlorine (OCPs) and organophosphorus (OPPs) pesticides, which are substances with high

persistence in the ecosystem that cause negative effects on the environment and human beings. OCPs such as endosulfan have a high persistence in aquatic ecosystems and low biodegradability and biomagnification in the tropical chain. They have also been associated with some types of cancer in humans (Xu, Liang, Guo, & Sun, 2018) but the role of endosulfan in leukemia cells has been unexplored. The aim of one of most representative study was to elucidate molecular mechanism of endosulfan-induced DNA damage response in human leukemia cells. They performed endosulfan exposure experiments in K562 cells with varying concentrations of endosulfan for 48 h and found that endosulfan lowered cell viability in a dose-dependent manner. Finding that are toxic compounds for most fish, and cause mass mortality (Naqvi & Vaishnavi, 1993).

On the contrary, OPPs are highly persistent in water and have eco-toxicological effects. According to Benotti et al. (2009) potential endocrine disrupting compounds (EDCs), the processes for treating drinking water are not effective in removing these pesticides and they can be found in treated and bottled water. Carcinogenic effects and lethal intoxication have been reported in humans (Biziuk, Przyjazny, Czerwmskl, & Wiergowski, 1996). The metabolites produced by pesticide degradation have attracted a significant amount of attention in recent years because they can be more dangerous and persistent than the active ingredients themselves (Geerdink, Niessen, & Brinkman, 2002; Koplin et al., 1998; Sinclair & Boxall, 2003). It is also indicated that, in the near future, the transformation products (TPs) rather than the parent compounds should receive most attention with a better understanding of matrix effects and eluent composition on the ionization efficiency of analytes being urgently required.

Globally, efforts have been made in recent years to control the indiscriminate use of pesticides, herbicides, and fungicides for comprehensive management of pests and diseases. However, residual amounts of these chemicals can still be found in water, soil, atmosphere and food, thus generating great concern for the population (Carvalho, Nhan, Zhong, Tavares, & Klaine, 1998; FAO, 2004). Most agrochemicals pose no toxicity to humans, animals, or the ecosystem. However, several studies have revealed multiple damages to the environment due to the frequent exposure of mixtures of active compounds which, although they are mostly found below the maximum residuality limits (LMRs), can interact with synergistic mixtures resulting in dangerous effects (Goujon et al., 2014; Tien, Lin, Chiu, & Chen, 2013) a triketone herbicide used to control dicotyledonous weeds in maize culture is rapidly photolyzed on plant foliage and generate two main photoproducts the xanthene-1,9-dione-3,4-dihydro-6-methylsulfonyl and 2-chloro-4-methyl benzoic acid (CMBA).

Intensive agriculture is currently conducted in Colombia, where onions are grown without crop rotation and with high demand for agrochemicals for pest control. However, in this country there are no studies that demonstrate the presence of persistent contaminants in crops or in onions on the market, and the impact of these pesticides on the environment and on human health are unknown. This reseach aims to characterise the use of agrochemicals in the study area and to monitor the use of organochlorine and organophosphorus pesticides during the production cycle to establish the residuality of pesticides present in onions (*Allium fistulosum*) cultivated in the middle upper basin of the Otún River, in the department of Risaralda, Colombia.

In this manuscript, we show that pesticides play an important role, but its indiscriminate use causes inadequate residual amounts to be present in onion (*A. fistulosum*). According to the MRL of the Codex Alimentarius (Codex Alimentarius FAO-WHO), it was found that 73.1% of the samples have residual exceeding the allowed limit of organochlorine pesticides, banned by the Rotterdam Convention by more than 40 times, posing a risk to human health and the ecosystem due to their chronic action.

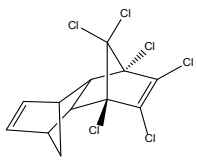
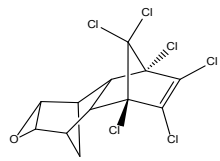
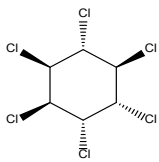
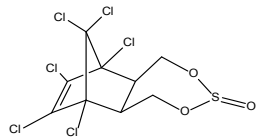
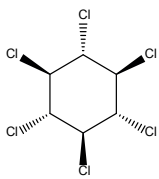
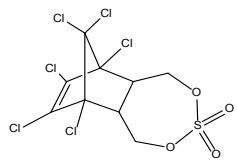
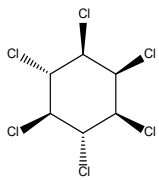
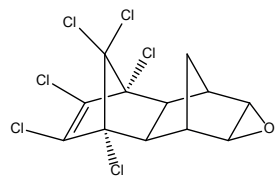
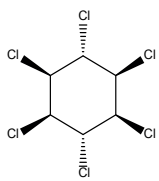
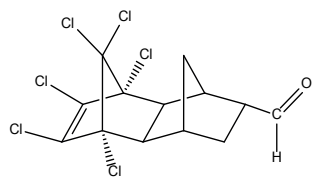
Our investigation constitutes a great contribution because accurate data is provided on the presence of highly toxic and prohibited pesticide residues, which represent a real risk to the population of developing countries, where studies are lacking due to analytical limitations and the scarcity of government control. This investigation shows that the obtained data serves as proof to develop strategies that allow a better control by governmental entities and to find alternatives to lower the exposure of humans and other living beings to these types of pollutants.

Materials and Methods

Chemicals and reagents

A standard of organochlorine pesticides Mix AB #1 32291 (Restek), organophosphorus pesticides Mix A 32277 (Restek) was used (Table 1 and 2). Acetone, petroleum ether, dichloromethane, and hexane HPLC-grade (Merck), anhydrous Na_2SO_4 (Sigma–Aldrich), Florisil® (Supelco) and 0.22 µm PTFE filters were used (Merck).

Table 1
Organochlorine pesticides structures

Compund	Structure	Compund	Structure
Aldrin		Dieldrin	
alpha-BHC		Endosulfan II	
beta-BHC		Endosulfan sulfate	
delta-BHC		Endrin	
gamma-BHC (Lindane)		Endrin aldehyde	

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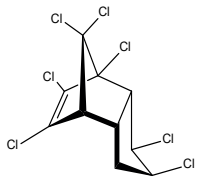
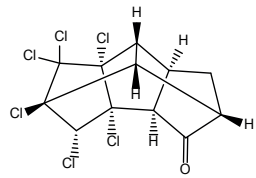
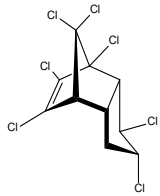
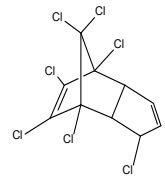
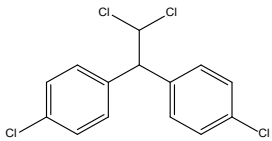
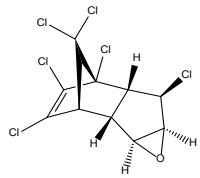
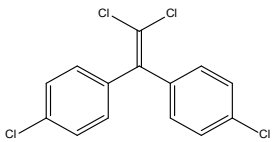
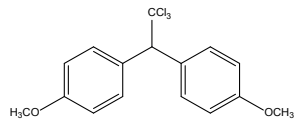
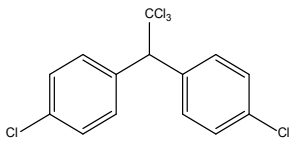
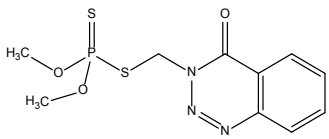
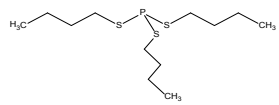
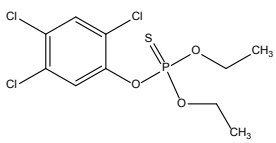
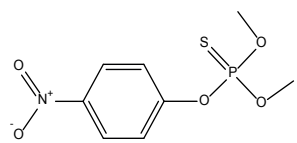
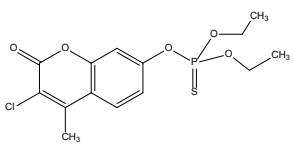
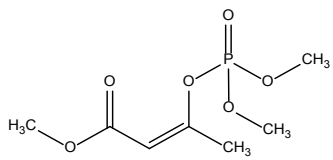
cis-Chlordane		Endrin ketone	
trans-Chlordane		Heptachlor	
4,4'-DDD		Heptachlor epoxide (isomer B)	
4,4'-DDE		Methoxychlor	
4,4'-DDT			

Table 2
Organophosphorus pesticides structures

Compund	Structure	Compund	Structure
Azinphos methyl		Merphos	
Chlorpyrifos		Methyl parathion	
Coumaphos		Mevinphos	

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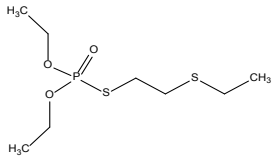
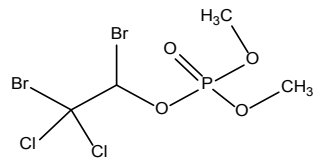
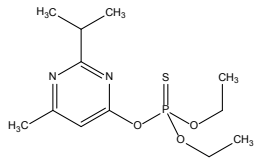
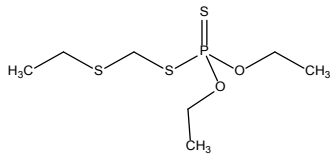
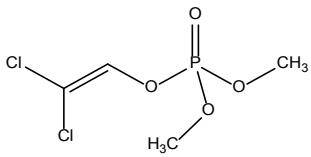
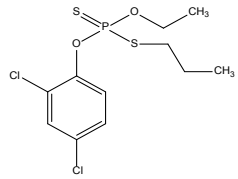
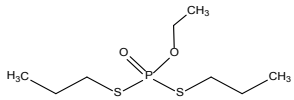
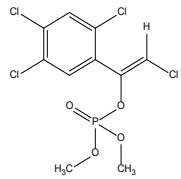
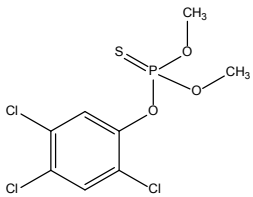
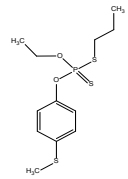
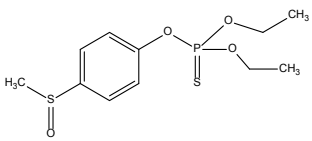
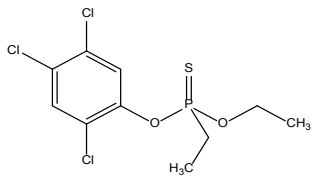
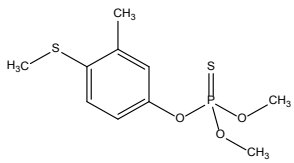
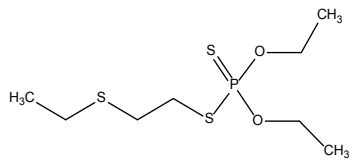
Demeton		Naled	
Diazinon		Phorate	
Dichlorvos (DDVP)		Prothiofos	
Ethoprophos		Stirofos (Tetrachlorvinphos)	
Fenchlorphos (Ronnel)		Sulprofos	
Fensulfothion		Trichloronate	
Fenthion		Disulfoton	

Vegetable material sampling collection

Samples 2 kg of junca onion (*A. fistulosum*) were taken from (*A. fistulosum*) crops in the upper–middle basin of the Otún River located at 4 ° 46'15 "N and 75 ° 36'59 "W on the Western flank of the Cordillera Central, in the department of Risaralda, Colombia. Beteween April to September of 2017, within a 314.25 Ha sown area distributed among four of the productive farms surveyed: El Eden, Alto Bonito; El Manzano and La Isabela (see Figure 1), where the samples were collected by monitoring the sample for six (6) months in a row.

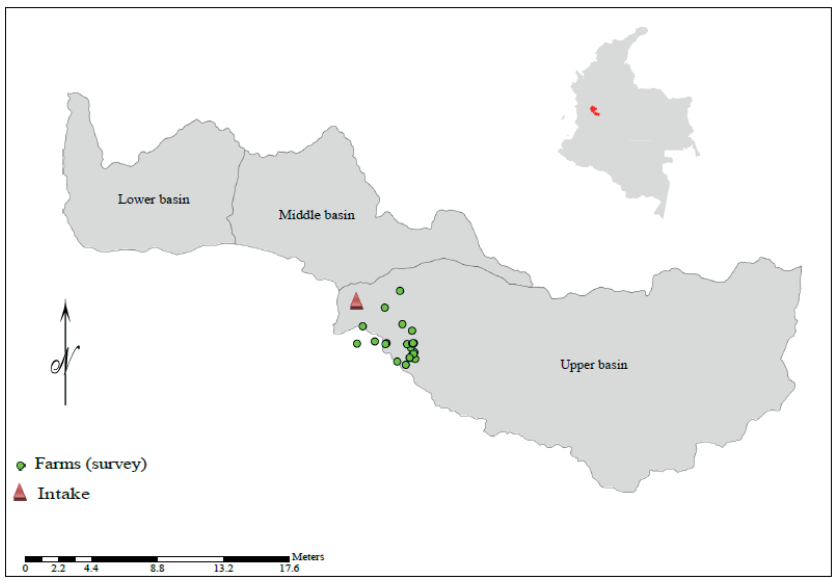


Figure 1. Location of surveyed properties, Otún river basin, Colombia, South America.

Sample preparation

Composite samples of 2.0 kg of plant material from each farm, were taken with the systematic random method following a previously established zig-zag point sampling parameter. The samples from each farm were transferred to the laboratory, where the adhered soil was removed. The weight was reduced to 1.0 kg by quartering, according to the methodology established by Dramiñski and Zagorzycki (1984). They were then stored in bags with an airtight seal 269.15 K for further testing.

Sample extraction and clean-up

According to the methodology described by Gamón, Lleó and Ten (2001), with some modifications: 2.5 g of the homogenised sample was taken, 5 mL of acetone was added at a 1:2 (m/v) sample solvent ratio and it was homogenised for 1 min. Subsequently, 10 mL of a 1:1 (v/v) mixture of dichloromethane petroleum ether was added and homogenised for 1 min. The mixture was transferred to a separation funnel and vigorously shaken for 2 min and centrifuged at 3500 rpm for 15 min. The supernatant was concentrated under reduced pressure at 308.15 K and then under a stream of nitrogen until dry and re-dissolved in 500 µL hexane.

The samples were cleaned using a column filled with solid phase of Florisil and anhydrous sodium sulfate at a 1:3 (m/m) ratios. Initially, the column was conditioned with 4 mL hexane, the sample was added and eluted with 9 mL a mixture hexane–acetone (9:1) (v/v), the extract was concentrated under a

stream of nitrogen, re-dissolved in 1 mL of hexane and filtered through a 0.22 µm PTFE membrane for further testing by gas chromatography coupled with mass spectrometry (GC-MS), according to the methodology adapted from (Ahmed, 2001; Gamón et al., 2001). The above process is shown in Figure 2.

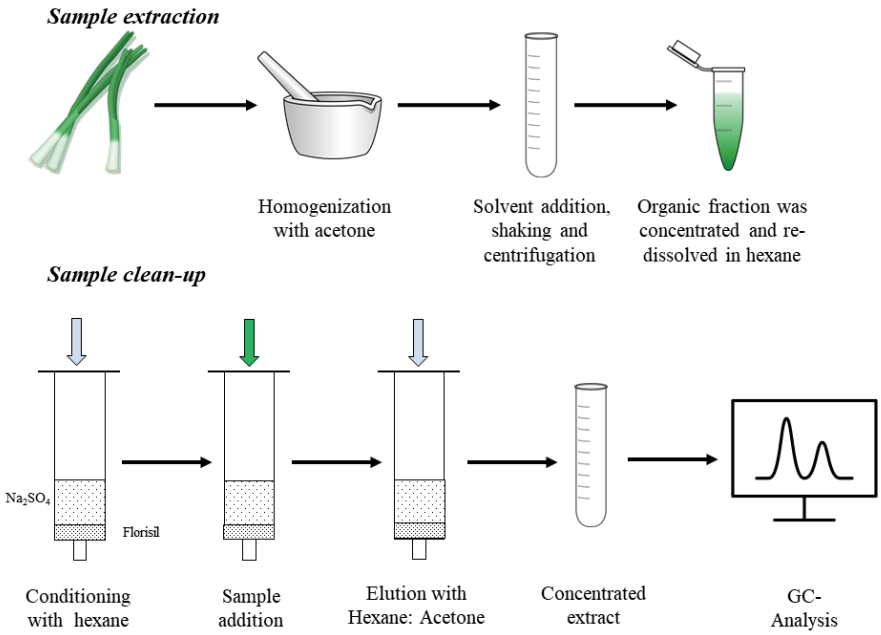


Figure 2. Sample extraction and clean-up process

Gas chromatography-mass spectrometry (GC-MS)

Determination of the target analytes was performed using a GC-2010 Plus gas chromatograph equipped with an AOC-20i+s automatic injection system,coupled to a QP-2020 mass spectrometer (Shimadzu, Kyoto, Japan), and a capillary column SH-Rxi-5Sil MS Crossbond® (30 m, 0.25 mm ID, 0.25 µm), using helium (purity 99.999%) as the carrier gas with a volumetric flow rate of 1.06 mL min^{-1}; the injection volume was 1 µL in splitless mode with a temperature of 523.15 K. All analytes were determined in a single injection. The GC oven was initially set at 383.15 K and increased to 423.15 K at 281.15 K min^{-1}, followed by a temperature ramp of 283.15 K min^{-1} to 473.15 K and held for 1 min; finally, it was increased to 533.15 K at 279.15 K min^{-1} and maintained for 16.50 min, for the total chromatographic run time of 27 min. The mass spectrometer was operated in electron impact (EI) mode at 70 eV, while the transfer line and the ion source temperatures were set at 533.15 K and 523.15 K, respectively. The MS system was programmed in selective ion monitoring (SIM) according to the conditions for any pesticide. The LabSolutions programm of GCMSSolution (Ver. 4 Shimadzu Corporation) was used for data acquisition, he presence of three ion fragments was used for compound identification, and retention time (R_t) was used for confirmation.

Preparation of standards and calibration curves

Calibration curves for testing organochlorine and organophosphorus pesticides were generated within regulations, with the maximum values allowed for onions according to the Codex Alimentarius (FAO, 2017) and the Environmental Protection Agency (EPA). A working standard solution was prepared

individually for each mixture of organochlorine and organophosphorus pesticides (stored at 277.15 K), respectively in triplicate, at a concentration of 1000 μg L^{-1} in their respective solvent and, from this solution, the standards for the construction of the calibration curve in a concentration range of 10, 50, 100, 300, 500, 700, and 1000 μg L^{-1} for organochlorine pesticides and 10, 50, 300, 500, 700 μg L^{-1} for organophosphorus pesticides.

LOQ and LOD

The following analytical performance parameters were determined, linearity, according to the calibration curve made for each pesticide, where the precision was calculated according to the relative standard deviation (RSD). Limit of detection (LOD) and limit of quantification (LOQ) calculated as the signal-to-noise ratio (S/N) of the lowest concentration that can be detected and quantified, respectively; and the recovery percentage was determined using a mixture of pesticides at 200 μg L^{-1}, process performed using a reference blank (uncontaminated onion sample), and three contaminated samples (1000 μL at 200 μg L^{-1}) for three days and in duplicate.

Statistical analysis

The survey was statistically processed by performing univariate descriptive analysis, factor analysis, conglomerate analysis and correspondence using the SPSS program, version 20 (IBM, USA). Acquisition of data from pesticide testing in junca onion samples was performed using the LabSolutions software from GC-MSSolution (Ver. 4, Shimadzu Co.). A descriptive analysis was conducted for the determination of pesticides in sample, using measures of central tendency and dispersion, with the SPSS program, version 20 (IBM, USA). The results obtained were compared with the current regulations.

Results and Discussion

Characterisation of agricultural practices

To establish the groups of pesticides used during the productive stage of junca onion (*Allium fistulosum*), a survey was conducted in 22 productive farms, of which 9.1% belong to growers associations and only 27.3% of the farmers confirm receiving technical assistance. It is noteworthy that 86% of the farms lack a certificate of good agricultural practices.

The results of the survey revealed that 70 different agrochemical inputs are used on the crop, of which 40 are used for pest control. 39.8% of the reported products are classified as highly toxic (category II), 6.8% are extremely toxic (category I) and 47.8% are moderately toxic (category III) and only 5.6% corresponds to slightly toxic products (category IV).

The most used products are Dithane ™ 5.6% (Mancozeb), Lorsban™ 5.6% (Chlorpyrifos), Fitoraz® 5.2% (Propineb); Antracol® 5.2% (Propineb) and Pyrinex® (4.8%) (Chlorpyrifos). The use of the following products was also reported: Abafed (Abamectin), Abasac (Abamectin), Acaramik (Abamectin), Actara® (Thiamethoxam), Aguila® WG (Metiram), Amistar® (Azoxystrobin), Amistar Top® (Azoxystrobin and diphenoconazole), Brestanid (Fentin hydroxide), Brigade® (Bifenthrin), Cantus® (Boscalid), CapsiAlil® (plants extract of Liliaceae and Solanaceae family), Carbendazim (Carbendazim), Cipertrin® (Cypermethrin), Cobrethane™ (Mancozeb), Connect® duo (Imidacloprid and Beta-cyfluthrin), Curacron® (Prophenophos), Cyromex® (Cyromazine and N-cyclopropyl-1,3,5-triazine-2,4,6-triamine), Daconil® 720 (Chlorothalonil), Decis® Fluxx (Deltamethrin), Diligent®720 (Metalaxil and mancozeb), Divino®EC (Diphenoconazole), Elosal® (Sulfur), Emerald® (Imidacloprid), Engeo® (Thiamethoxam and lambdacyhalothrin), Estoque® (Cypermethrin), Evofarm® (Pyrazosulfuron etil), Exalt™ (Spinetoram, spinosyn J and spinosyn

L), Fitoraz® (Propineb), Forum® (Dimetomorf), Fulminator (Cypermethrin and prophenophos), Geminis® (Imidacloprid and lambda-cyhalothrin), Karate® zeon cs (Lambda-cyhalothrin), Kasumin® (Kasugamycin) and Lannate®SL (Metomil).

The products reported by farmers are used for 249 different types of treatments for pests and diseases. The main groups of pesticides are dithiocarbamates, organophosphates and organochlorines, according to the frequency of use. The survey made it possible to establish a sampling plan, according to the analytical possibilities, for the monitoring of organochlorine and organophosphorus pesticide groups in junca onion (*Allium fistulosum*) in four representative plots, which belong to farmers who sell fresh onions. There are also medium and large producers of junca onion, who perform the primary transformation, have defined marketing channels and are also marketers. The selected properties have the names: El Edén, Alto Bonito; El Manzano and La Isabella.

Calibration curves for organochlorine pesticides (OCPs)

Table 3 reports retention times between 11.285 ± 0.002 min and 22.115 ± 0.002 min for each organochlorine compound and these experiments presented satisfactory separation. For the calibration curve, the equation of the line and the correlation coefficients with values between 0.9814 and 0.9998 are reported, indicating good linearity for each curve. As shown in Table 3, the LOD reported for each organochlorine pesticide was between 0.469 and 7.148 μg kg^{-1} and LOQ between 1.336 and 18.535 μg kg^{-1}. In terms of precision, values such as the relative standard deviation (RSD) were reported between 0.814% and 1.006%.

Table 3
Data for the calibration curve of organochlorine pesticide

Pesticide	Retention time (min)	Linearity (R^2)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	%RSD
alpha-BHC	11.285	0.998	1.889	4.977	1.045
beta-BHC	11.893	0.988	2.413	6.258	0.987
gamma-BHC	12.164	0.995	1.787	4.655	0.814
delta- HC	12.861	0.993	2.527	6.553	0.987
Heptachlor	14.212	0.987	1.829	4.742	0.987
Aldrin	15.259	0.988	1.459	3.782	0.987
Heptachlor Epoxide (B Isomer)	16.358	0.995	2.968	7.696	0.987
cis-Chlordane	17.052	0.999	1.878	5.261	1.044
trans-chlordane	17.443	0.999	1.739	4.729	1.044
4.4'-DDE	18.032	0.995	0.765	2.140	1.034
Dieldrin	18.263	0.996	1.044	2.844	1.044
Endrin	18.889	0.981	5.563	14.426	0.987
Endosulfan II	19.183	0.992	7.148	18.535	0.987
4.4'-DDD	19.275	0.998	0.469	1.336	1.006
Endrin aldehyde	19.615	0.998	1.549	4.333	1.034
Endosulfan sulfate	20.320	0.995	4.862	13.259	1.048
4.4'-DDT	20.414	0.989	1.044	2.707	0.987

continue

continuation

Endrin ketone	21.789	0.998	2.042	5.294	0.987
Methoxychlor	22.115	0.994	0.514	1.464	1.006

Rt: Retention time
LOD: limit of detection
LOQ: limit of quantification
RSD: relative standard deviation.

The method developed in this research is comparable with the study conducted by Li et al., (2014) for the determination of organochlorine pesticides (OCPs) in edible vegetable oil samples, the GC-MS method with LOQ and LOD reports between 0.14 and 2.45 μg kg^{-1} and between 0.06 and 0.74 μg kg^{-1}, respectively, and RSD reproducibilities between 0.8% and 27.3%. Also, Arias, Arrubla & Giraldo (2018) reported a method GC MS for determination of organochlorine and triazoles pesticides in export-type green coffee, indicating that accuracy and precision (% RSD), in all cases, were higher than 94% for five levels of concentration. The correlations of the calibration curves oscillated between 0.996 and 0.999, y for linear working ranges between 0.005 and 0.800 mg kg^{-1}. Detection limits were in all cases lower than 0.025 mg kg^{-1}, in a way that complied with legislative requirements. Recovery rates fluctuated between 71.9% and 93.9%, with dispersions below 9.13%.

The method developed in this research provides the appropriate levels of detection and quantification according to the MRL reported by Codex Alimentarius, in addition to having coefficients of variance of less than 2%.

Calibration curve for organophosphorus pesticides (OPPs)

Table 4 shows the retention times for each organosphosphorated compound between 5.093 ± 0.015 and 26.272 ± 0.003 min. According to the calibration curve in Table 4, the equation of the line is reported and a correlation coefficient between 0.9720 and 0.9976 indicates good linearity for each curve and that the concentration for each compound area of interest within the constructed curve will have an influence ≥ 97.2% on the area of each one.

Table 4
Data for the calibration curve of organophosphorus pesticide mixture

Pesticide	Retention time (min)	Linearity (R^2)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	%RSD
Dichlorvos	5.093	0.983	0.110	0.311	1.064
Mevinphos	7.607	0.986	0.120	0.340	1.046
Demeton O & S	9.990	0.986	0.412	1.169	0.918
Ethoprophos	10.306	0.990	0.379	1.073	0.882
Naled	10.586	0.983	0.619	1.754	1.348
Phorate	11.088	0.988	0.107	0.302	1.076

continue

continuation

Diazinon	12.319	0.991	0.275	0.779	0.855
Disulfoton	12.671	0.990	0.118	0.335	1.055
Methyl parathion	13.861	0.974	0.496	1.407	1.059
Fenchlorphos (Ronnel)	14.189	0.998	0.232	0.658	0.892
Chlorpyrifos	15.136	0.989	0.570	1.617	1.224
Fenthion	15.028	0.991	0.197	0.559	0.925
Trichloronate	15.557	0.990	0.214	0.606	0.903
Stirofos (Tetrachlorvinphos)	17.076	0.977	0.263	0.745	0.847
Prothiofos	17.767	0.993	0.552	1.566	1.181
Merphos	18.059	0.986	0.366	1.036	0.868
Fensulfothion	19.014	0.985	0.849	2.407	0.859
Sulprofos	19.733	0.991	0.268	0.760	0.833
Azinphos-methyl	23.268	0.972	0.304	0.862	1.167
Coumaphos	26.272	0.992	0.546	1.548	1.064

Rt: Retention time
LOD: limit of detection
LOQ: limit of quantification
RSD: relative standard deviation.

The LOD reported for each organophosphorus pesticide was between 0.107 and 0.849 μg kg^{-1} and the LOQ between 0.302 and 2.407 μg kg^{-1}. In terms of the accuracy, the relative standard deviation (RSD) values were found between 0.833 and 1.348%. The method developed in this investigation presents levels of quantification in equivalent ranges for chromatographic systems with specific detectors, such as the nitrogen–phosphorus detector (NPD), reported by Hajjo, Afifi, and Battah (2007) for testing the OPPs in vegetable matrices with LOQ between 0.8 and 50 μg kg^{-1}.

Standardization of the method of extraction for organochlorine and OPPs

Recovery for organochlorine and Organophosphorus pesticides

A sample of 2.5 g junca onion (*A. fistulosum*) was tested, which was contaminated with a standard mix of organochlorine and organophosphorus pesticides, both at a concentration of 80 μg kg^{-1} and it was used a reference blank (uncontaminated onion sample). We proceeded to the extraction of the contaminated samples and florisil was used for the interferences, such as the green pigments and sulfur compounds of the onion, and for the cleaning the extracts, eliminating any residual component that could interfere with the analysis of GC-MS (Alamgir et al., 2013).

As shown in figures 3 and 4, were obtained recovery percentages between 46.32% ± 3.28 - 118.67% ± 9.10 for organochlorine pesticides and between 58.087% ± 1.55 - 99.24% ± 2.61 for organophosphorus pesticides in samples of junca onion (*A. fistulosum*). Similar percentages were obtained in the study carried out by Ozcan (2016) who reported values between 83% to 104% recovery. Thirty-nine pesticides were evaluated with the proposed extraction method and it was possible to detect thirty pesticides, fifteen organochlorine pesticides and eleven organophosphorus pesticides. All analytes were determined in a single injection.

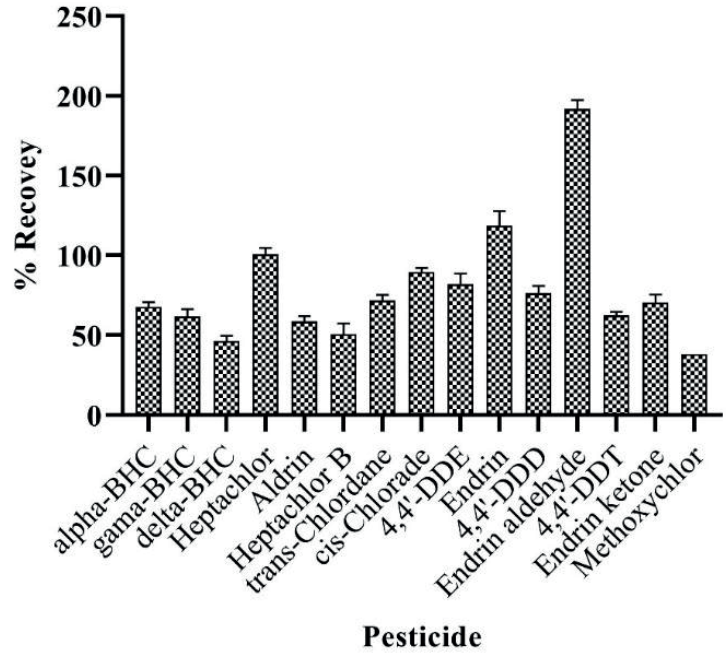


Figure 3. Recovery values of organochlorine pesticides (OCPs) in contaminated onion (Allium fistulosum) samples.

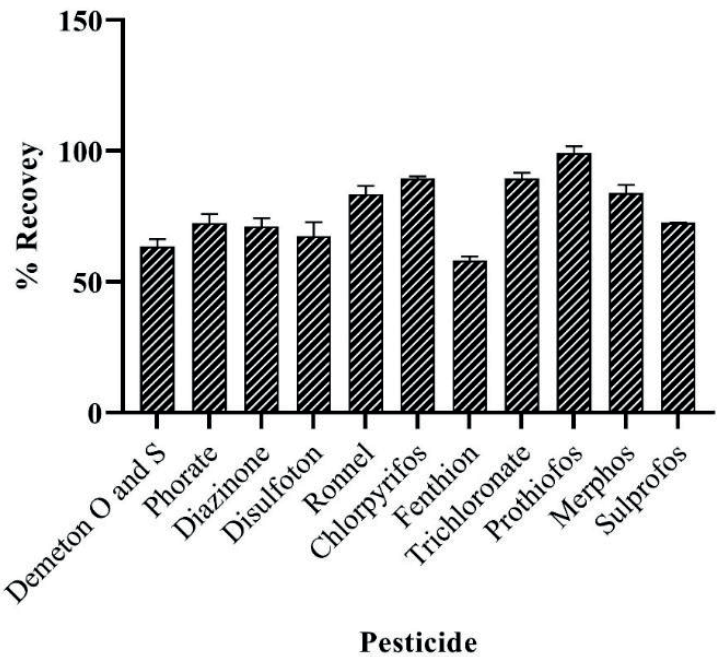


Figure 4. Recovery values of organophosphorus pesticides (OPPs) in contaminated junca onion (*Allium fistulosum*) samples.

This validation results are comparable with the reports by Skovgaard et al. (2017), who implemented a GC-MS method for the analysis of pesticides in crop samples (lettuce, onion and potatoes), with a detection limit of 3 μg kg^{-1}, a limit of quantification of 10 μg kg^{-1} and a recovery between 70% and 120%. Guan et al., (2014) reported the validation of the LC-MS / MS method for the determination of pesticides in onion, garlic and leek, with a detection limit between 1 to 10 μg kg^{-1} and a recovery percentage between 70.1% and 109.7%. Similarly, Acosta Rodrigues, Souza Caldas, and Primel (2010) implemented a LC-MS/MS method for these matrix and compounds, with recovery percentages between 70% and 120%.

Although LC-MS / MS methods allow achieving high sensitivity, selectivity and robustness to monitor multiple pesticide residues in fruits and vegetables (Kruve, Künnapas, Herodes, & Leito., 2008; Soler, Mañes, & Picó, 2005). The GC-MS can be used, for the monitoring of specific compounds such as organochlorine and organophosphate pesticides (Alder, Greulich, Kempe, & Vieth, 2006) being an accessible and less expensive technique (Scherbaum et al., 2008).

GC-MS represents a good option because its universal ionization technique, allows comparison with a big spectrum library and provide enough information on the analyte structure without the need to derivatization (Stachniuk & Fornal, 2016).

Analysis of organochlorine (OCPs) and organophosphorus (OPPs) in samples of junca onion (A. fistulosum)

Samples from four production centres were tested, which were collected during six consecutive months between the winter and summer seasons. As shown in Tables 5 and 6, the presence of ten compounds was found in the OCPs group and the presence of nine compounds was reported for the OPPs group.

The organochlorine pesticide Endrin ketone showed a high concentration of up to 2045.99 μg kg^{-1} of sample. Particularly the El Edén farm, in sampling one, reported the highest concentration of endrin with a value of 1618.42 μg kg^{-1} of sample and the Alto Bonito farm reported the lowest content of organochlorinated pesticide compound (table 5).

According to the results, the concentrations obtained were higher than those reported for OCPs and OPPs in onion cultivated worldwide. A study performed by Quintero et al., (2008) in Venezuela, report the presence of methamidophos in concentration between 10 and 40 μg kg^{-1} and diazinon in a range between 10 and 70 μg kg^{-1} in green onion. In the research carried out by Sapbamrer and Hongsibsong (2014) for spring onion samples obtained in Thailand, they report the presence of OPPs such as chlorpyrifos with 18 μg kg^{-1} and diazinon with μg kg^{-1}. For onion samples analized in Turkia, Ozcan (2016) reported 16 OCPs varying between 3.33 μg kg^{-1} for heptachlor epoxide to 36.4 μg kg^{-1} for 4.4'-DDD, being the compound with the highest concentration. Meanwhile, studies carried out in Ethiopia by Sishu et al., (2020), reported the presence of α-endosulfan with concentrations greater than 50 μg kg^{-1} and β-endosulfan with maximum levels of 41.8 μg kg^{-1}. Exceptionally in Bolivia, Skovgaard et al. (2017) did not detect the presence of OPPs for onion crops.

Tables 7 and 8 report the maximum residue limit of organochlorine and OPPs, respectively, in some fruits and vegetables. It is important to note that for all the samplings conducted, at least one organochlorine pesticide exceeding the MRL is seen. The most prominent is endrin and its degradation products (endrin aldehyde and endrin ketone) have an MRL of 50 μg kg^{-1} in cucurbits, such as cucumber, melon and watermelon (reference product), and the acceptable daily intake is 0.2 μg kg^{-1} body weight (FAO, 2017). It can be observed that the concentrations reported in this study exceed the MRLs by more than 40 times in comparison with what was reported by the Codex Alimentarius.

From the viewpoint of organophosphorus pesticides, methyl parathion is reported as a banned substance with concentrations of up to 15. They do not exceed the MRLs reported in the Codex Alimentarius, but it is noted that the trichloronate that reported a high concentration in this study is not referenced in the Codex Alimentarius. However, the risk or possible impact that this pesticide generates when presenting a high concentration should not be ruled out. Methyl parathion according to the Environmental Protection Agency of the USA (EPA) reports an MRL of 3000 $\mu g\ kg^{-1}$ for branch onion (FAO, 2017).

This research, which provides information on the habits of pesticide use by farmers during the cultivation of onions in the Risaralda region in Colombia, reveals the high levels of residuals found for OCPs and OPPs (between LOQ and 2046 ppb) for all samplings (100%), in correspondence with the low percentage of farmers receiving technical assistance (≤27.3%) and the use of a large number of products (40), reported for pest control.

The presence of pesticides banned or severely restricted by the Rotterdam agreement, which were not reported in the surveys, such as DDT, heptachlor, lindane, endrin, endosulfan and methyl parathion, is evident in all the samples (Rotterdam Convention. On the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade, 1998). This situation is alarming due to the danger that these restricted products pose to human health and the environment. It is necessary to emphasise the importance of education to farmers for the proper use of pesticides, in terms of the choice of product and the correct dosage to avoid contamination of the products which are marketed. Investigations from Giraldo et al., (2020) in the region of Risaralda, Colombia stand out, from where natural extracts have been used to fight the onion pests as an alternative to synthetic pesticides, for example against *T. tabaci*, the *A. muricata L.* extract showed LC_{50} = 82.93 mg L^{-1}, the onion essential oil showed LC_{50} = 335.29 mg L^{-1}, and the onion hydrolate showed LC_{50} = 2348.84 mg L^{-1}. The combinations of extracts reached mortality rates between 50% and 72.62%, which are promising results as alternatives for the comprehensive management of the onion pests.

Table 5.
Organochlorine pesticide content in samples of junca onion (A. fistulosum)

		Mean levels of pesticide residues per sampling (µg kg^{-1})					
Class Name	**OCPs**	**1**	**2**	**3**	**4**	**5**	**6**
El Edén	4,4'-DDT	-	-	91.43 ±0.14%	376.95 ±0.01%	-	181.33 ±0.02%
	alpha-BHC	-	-	14.08 ±2.6%	-	17.01 ±0.72%	-
	Dieldrin	-	-	-	-	-	117.84 ±0.19%
	Endrin	1618.42 ±0.01%	194.48 ±0.19%	-	39.06 ±0.54%	-	738.61 ±0.07%
	Endrin aldehyde	763.66 ±0.02%	25.83 ±0.33%	-	21.96 ±4.21%	38.41 ±0.05%	-
	Endrin ketone	-	497.56 ±0.09%	1231.98 ±0.01%	1443.72 ±0.06%	176.11 ±0.02%	1526.61 ±0.01%
	Heptachlor	18.84 ±7.51%	-	-	-	-	-
Alto Bonito	4,4'-DDD	-	-	-	-	-	29.84 ±0.65 %
	4,4'-DDT	48.84 ±1.15%	-	130.55 ±1.08%	333.86 ±0.04%	-	-
	alpha-BHC	9.83 ±1.98%	-	-	21.30 ±6.64%	-	55.88 ±0.29%
	Endosulfán II	-	-	-	-	-	503.63 ±0.01%
	Endrin	254.89 ±0.02%	173.78 ±0.06%	127.75 ±0.02%	457.25 ±0.31%	-	-
	Endrin aldehyde	54.17 ±0.83%	51.97 ±0.53%	40.30 ±0.45%	37.95 ±0.38%	28.89 ±1.12%	-
	Endrin ketone	84.56 ±0.18%	635.47 ±0.002%	889.59 ±0.005%	1714.07 ±0.001%	248.19 ±0.003%	-
El Manzano	4,4'-DDT	-	-	134.2 ±0.01%	366.34 ±0.01%	-	252.9 ±0.03%
	alpha-BHC	-	16.53 ±8.56%	18.10 ±3.40%	-	-	-
	Dieldrin	9.53 ±7.66%	-	-	-	-	155.24 ±0.17%
	Endrin	104.90 ±0.01%	250.31 ±0.05%	212.10 ±0.67%	-	-	459.39 ±0.04%
	Endrin aldehyde	48.94 ±1.03%	7.66 ±1.14%	24.98 ±5.66%	-	13.91 ±0.32%	-
	Endrin ketone	103.43 ±0.03%	572.41 ±0.03%	1417.31 ±0.001%	980.22 ±0.004%	263.33 ±0.004%	1442.3 ±0.01%
	Heptachlor	-	-	-	-	-	-
	Methoxychlor	9.91 ±1.92%	-	-	-	-	-

La Isabella	4,4'-DDT	-	15.73 ±8.99%	-	438.35 ±0.03%	-	284.21 ±0.05%
	alpha-BHC	-	-	20.41 ±6.93%	33.01 ±0.01%	-	25.90 ±5.46%
	Dieldrin	565.63 ±0.02%	-	-	-	-	-
	Endrin	-	178.38 ±0.04%	141.25 ±0.04%	-	52.08 ±0.92%	276.53 ±0.51%
	Endrin aldehyde	34.41 ±0.45%	24.13 ±5.86%	57.32 ±0.07%	50.62 ±0.31%	-	-
	Endrin ketone	120.70 ±0.20%	1024.79 ±0.002%	1741.53 ±0.001%	-	574.33 ±0.02%	2046.00 ±0.001%
	Heptachlor	5.88 ±8.39%	-	-	-	-	-

The results were expressed as mean ± %RSD
(-) Not detected.

Table 6
Organophosphorus pesticide content in samples of junca onion (A. fistulosum)

		Mean levels of pesticide residues per sampling (µg kg^{-1})					
Class Name	**OPPs**	**1**	**2**	**3**	**4**	**5**	**6**
El Edén	Azinphos-methyl	16.57 ±1.45%	-	-	-	-	-
	Disulfoton	9.44 ±0.37%	9.88 ±0.53%	-	-	9.76 ±0.02%	-
	Ethoprophos	-	-	16.90 ±7.40%	-	-	-
	Methyl parathion	12.98 ±0.32%	-	-	-	-	-
	Merphos	-	9.88 ±0.53%	-	-	-	-
	Trichloronate	7.54 ±1.88%	-	-	8.60 ±0.60%	8.06 ±0.39%	-
Alto Bonito	Chlorpyrifos	-	-	-	-	-	22.31 ±0.19%
	Methyl parathion	-	-	14.73 ±0.89%	-	-	-
	Merphos	11.85 ±0.50%	-	-	-	-	-
	Trichloronate	-	7.63 ±18.53%	-	8.79 ±0.53%	-	239.10 ±0.01%
El Manzano	Disulfoton	50.91 ±0.07%	-	-	-	-	-
	Merphos	15.00 ±0.22%	-	-	11.73 ±0.11%	11.10 ±0.06%	-
	Trichloronate	-	-	-	8.72 ±0.10%	7.68 ±1.17%	-
La Isabella	Azinphos-methyl	14.83 ±0.21%	-	-	-	-	-
	Chlorpyrifos	9.17 ±15.42%	-	-	-	-	-
	Coumaphos	9.48 ±14.92%	-	-	-	-	-
	Methyl parathion	-	-	-	12.87 ±0.32%	-	-
	Prothiofos	-	-	-	8.40 ±0.53%	-	-
	Merphos	10.72 ±0.06%	-	-	11.18 ±1.13%	-	-
	Trichloronate	16.57 ±0.01%	-	-	-	-	-

The results were expressed as mean ± %RSD
(-) Not detected.

Table 7
Organochlorine pesticides, commercial products, maximum residue limit (MRLs) and acceptable daily intake (ADI) (FAO, 2017)

Pesticide	Commercial Product	Maximum residue levels, MRLs (µg kg^{-1})	Reference agricultural product	Acceptable daily intake (ADI) (µg kg^{-1} body weight)
4,4'-DDD	Rothane, TDE	200	Carrot	10
4,4'-DDT		200	Carrot	10
alpha-BHC	BHC Lindane	10	Corn and cereal grain	5
Dieldrin	Heod, Dielorex, Octalox	50	Bulb Vegetables	0.1
Endosulfan II		50	Sweet potato, Potato	6
Endrin	Nendrina (active compound and its degradation products)	50	Cucurbitaceans (Cucumber, melon, watermelon)	0.2
Endrin aldehyde			----------	
Endrin ketone			----------	
Heptachlor	Vesicol	20	Cereal grain and soy	0.1

Table 8
Organophosphorus pesticides, commercial products, maximum residue levels (MRLs) and acceptable daily intake (ADI) (FAO, 2017)

Pesticide	Commercial Product	Maximum residue levels, MRLs (µg kg^{-1})	Reference agricultural product	Acceptable daily intake (ADI) (µg kg^{-1} body weight)
Azinphos-methyl		500	Vegetables	30
Chlorpyrifos	Lorsban, Latigo, Pyrinex	10 a 2000	Vegetables	10
Disulfoton	Dysiston, Dithidemeton, Disyston, Ditio-systos	200	Beets, Beans, Barley	0.3
Ethoprophos	Mocap, Prophos, Ethoprophos	50	Potato, Sweet Potato and Sweet Pepper	0.4
Methyl parathion		3000	Spices, Roots and Rhizomes	3

Conclusion

This study evaluates the current situation of the degree of contamination by organochlorine and organophosphorus pesticides in fresh onion ready to be commercialised in Risaralda, Colombia, South America. A greater presence of organochlorine pesticides banned or severely restricted by the Rotterdam agreement, such as 4,4'-DDT, was reported in samples of junca onion (*A. fistulosum*), with concentrations up to (221.22 μg kg^{-1} of sample), endrin (469.23 μg kg^{-1} of sample) and its degradation products which exceed the MRL for plant samples more than 40 times, reported by the Codex Alimentarius. The OPPs did not exceed the MRLs, highlighting the high concentration of trichloronate (239.10 μg kg^{-1} of sample), which is not reported in the Codex Alimentarius. Products that do not comply with the regulatory requirements represent a risk to human health, due to their chronic action. It is necessary to monitor other pesticides that were not included in this investigation and it is evident that the obtained data serves as proof to develop strategies that allow a better control by governmental entities and to find alternatives to lower the exposure of humans and other living beings to these types of pollutants.

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